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WITH FORTY-SIX PLATES AND ONE HUNDRED AND SEVENTY TEXT FIGURES

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#### ERRATA, VOLUME XIII

Page 27, 4th line from bottom. For *anomala*, read *anomalus*.

Page 194, 8th line from bottom. For *media*, read *Medium*.

Page 458, 1st line below Table 33. For 439, read 445.

Page 609, text figure 1, middle column. For *Quarternaria*, read *Quaternaria*.

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JANUARY, 1926

No. 1

## CYTOLOGICAL STUDIES IN THE CUCURBITACEAE

### I. MICROSPOROGENESIS IN *CUCURBITA MAXIMA*

EDWARD F. CASTETTER

(Received for publication February 2, 1925)

It seems strange that a genus so interesting and so economically important as *Cucurbita* should have received so little attention cytologically as has previously been given it. The most recent cytological work on the genus is that by Kirkwood (10) who has described and figured the development of the megaspores, the embryo sac, and the endosperm in *Cucurbita Pepo*. His paper contains a review of the earlier literature on megasporogenesis, fertilization, and pollen-tube growth in *Cucurbita* and allied genera.

The work done on microsporogenesis in the genus is surprisingly meager, and, although done with living material before the days of modern technique, is quite accurate. Mirbel (14) early described and figured the general features of pollen-mother-cell development, tetrad-formation, and pollen-grain differentiation in *Cucurbita Pepo*, and Nägeli (15) later studied the formation of the microspores and particularly the differentiation of the exine and intine and the relation of the latter to the growth of the pollen tube in the same species. Kirkwood (11) states that he studied the principal features in the development of the microspores of *Cucurbita Pepo* and *Cucurbita moschata*, but he neither describes nor figures any stages.

#### MATERIALS AND METHODS

Realizing the undesirability of working with commercial material in a genus in which there is so much intercrossing between varieties, the writer secured a pure line of Hubbard squash known as line no. 270 from Cummings (4), who developed it by inbreeding. Material was grown both in the greenhouse and in the field, and young flowers were taken from both sources. Material was fixed in one percent chrom-acetic acid to which were added ten drops of one percent osmic acid per 50 cc. of solution. Other material fixed in Bouin's fluid was found especially fine for stages for counting chromosomes. The material was imbedded in paraffin and cut from 3 to 10  $\mu$  thick. Some sections were stained with Heidenhain's iron-alum haematoxylin, others with Delafield's haematoxylin after being treated with four percent iron

alum for one hour. Others, stained with safranin and gentian violet, were found very useful in the study of cytokinesis. Living material was also used in the study of the mother-cell wall in its relation to furrowing.

The post-synizetic stages leading to the formation of the chromosomes in early diakinesis are very unusual, and the writer has reserved a consideration of them for a separate paper. The present paper will, therefore, begin with the origin of the multipolar spindle.

Farr (5) has emphasized the strong tendency which has existed for many decades among investigators to regard the division of the pollen mother cell in the higher plants as occurring by a process of cell-plate formation. His papers (5-8), which summarize the literature on the subject, show that in the higher plants the division of the pollen mother cell in forming the microspores is usually brought about by a process of furrowing and that no cell plate is formed, or at best only sporadic attempts occur to form cell plates. He (5) thinks that in the monocotyledons there are very few authentic cases of pollen-mother-cell division by the cell-plate method, although there are many fragmentary statements and drawings indicating that cell plates do occur. In the dicotyledons he considers the evidence of division by cell plates still more fragmentary, and states that "it appears that in no instance is the evidence conclusive that quadripartition of the pollen mother cells of any dicotyledon is effected by means of cell plates." In his paper on *Nelumbo lutea* (8), he refers to a paper by Lubimenko and Maige (12) who studied cytokinesis in *Nymphaea alba* and *Nuphar luteum*. For *Nymphaea* they describe and figure ephemeral, yet distinct, cell plates on the heterotypic and homoeotypic spindles, although they found that these plates do not extend to the plasma membrane. They believe that, after the cell plates and spindle fibers are resorbed to be used in rebuilding the nuclei, new spindle fibers originate from the cytoplasm. New cell plates, which in their opinion effect the division of the pollen mother cell, now appear on these spindles. Farr (8) regards this behavior in *Nymphaea* as without precedent in the literature and has, therefore, investigated the allied species, *Nelumbo lutea*, in which he found a transitory cell plate present in the heterotypic division extending only to the limits of the central spindle. He found no evidence of cell plates during the homoeotypic mitosis, and thinks that Lubimenko and Maige and other workers have mistaken thin centripetal extensions of the thick mother-cell wall for cell plates.

#### MULTIPOLAR SPINDLE AND HETEROTYPIC MITOSIS

Just previous to diakinesis the cytoplasm immediately surrounding the nucleus becomes densely granular, forming a rather compact region around the nucleus; this region has been referred to by several workers as the perinuclear zone. As the nuclear membrane disappears, numerous beaded fibers of nuclear origin appear in the nuclear cavity. These fibers form a web in which the chromosomes are seen. This fibrous web soon pro-

jects out into three or more cones (Pl. I, fig. 1) constituting the multipolar spindle, the fibers of which gradually become arranged in two groups to form the bipolar spindle. A somewhat similar method of origin of the multipolar spindle has been described in *Larix* by Allen (1), and recently by Santos (16) in *Elodea*. The pollen mother cell is at this stage surrounded by a homogeneous wall (fig. 1) which originates from the protoplast of the mother cell during early diakinesis, apparently by secretion. This wall is composed of callose, as has been determined by its microchemical reactions, and is quite similar to the wall of the pollen mother cell of *Melilotus alba* previously studied by the writer (3). No lamination is evident on the callose wall in *Cucurbita* at this stage. The chromosomes now become arranged on the heterotypic spindle, and sections cut at right angles to the spindle in the region of the equatorial plate, before the chromosomes divide, clearly show the number of bivalent chromosomes to be 20 (fig. 3). The writer's preparations show numerous mother cells at this stage in which the number of bivalent chromosomes is unquestionably 20. The double nature of the chromosomes at this stage, however, is not evident. Figure 4 is a view across the spindle of a root-tip cell showing 40 somatic chromosomes. Although the chromosomes are very small, they can be counted with ease during meiosis, since they do not clump together but stand well apart, especially when fixed with Bouin's fluid. More difficulty is encountered in making counts in the root tip, however, on account of the smaller size and double number of the chromosomes. Very few chromosome counts have been made on genera of the Cucurbitaceae, the only ones recorded being 16 (haploid) for *Micrampelis lobata* by Kirkwood (11); 10 (haploid) for *Bryonia alba* by Bönicke (2); 10 (haploid) for *Bryonia dioica* by Strasburger (17), and 24 (diploid) for *Cucurbita Pepo* by Lundegårdh (13).

Each heterotypic chromosome now separates into two univalent chromosomes (fig. 2), which gradually move toward the poles. During late anaphase the chromosomes become closely massed together and begin to fuse, forming an anastomosed chromatin mass which soon becomes surrounded by a nuclear membrane. At this stage the spindle shows a lighter region in the vicinity of the equator, and a distinct cell plate appears (fig. 5) which extends only across the central spindle. This cell plate is ephemeral and very soon disappears. The anastomosed chromatin mass in each nucleus now breaks up into clumps which a little later are often seen to be in pairs (fig. 6). Each pair is a split univalent chromosome just prior to the initiation of the homoeotypic division.

#### HOMOEOTYPIC MITOSIS

The homoeotypic spindles are now formed and are parallel (fig. 7), at right angles, or occasionally intermediate. Figure 8, Plate II, shows the spindles at right angles. On the left of the figure the univalent chromosomes are shown on the spindle, but, as in the same stage of the heterotypic division,



there is no evidence of a split in the chromosomes. The section was cut at right angles to the long axis of the other spindle, through the equatorial plate, before the chromosomes began to divide. Twenty univalent chromosomes are distinctly seen.

The chromosomes on each spindle now separate and the members move to the poles, where they fuse together more or less completely and become surrounded by a membrane (Pl. I, fig. 7), then fragment into small clumps (Pl. II, figs. 9, 10) as in the same stage of the heterotypic division. At this stage a distinct cell plate appears at the equator of each of the homoeotypic spindles. Just as in the heterotypic division, these cell plates never extend beyond the limits of the spindle and thus never reach the plasma membrane. These plates remain for only a short time and then disappear. The cell plates on both the heterotypic and the homoeotypic spindles seem to leave in their paths at the time of their disappearance a band of densely granular material which is doubtless a product of the disintegration of the spindles, and which soon becomes entirely dissipated.

#### CYTOKINESIS

A study of living material stained with resorcin blue shows that the callose mother-cell wall, soon after the heterotypic division has taken place, is distinctly laminated. This lamination is not evident in fixed material. Immediately surrounding the protoplast a new wall is now formed, apparently by secretion from the mother cell. This *special wall*, which is also composed of callose, stains a deeper blue and is more refractive than the outer wall. The writer (3) recently described a similar special wall in the pollen mother cell of *Melilotus*. When the stage represented by figure 13 is reached, the outer laminae have entirely broken down and only the special wall surrounding the microspores is seen.

When the spindles occur at right angles to each other (fig. 8), the daughter nuclei assume a tetrahedral arrangement, lying as far apart from each other as possible. The homoeotypic spindles and cell plates now disappear and the cytoplasm becomes more vacuolate, particularly at the region midway between each pair of nuclei (fig. 9). The spherical protoplast assumes the shape of a tetrahedron with four equidistant nuclei, one near each corner. Each side of the tetrahedron is practically an equilateral triangle with a slightly convex face and distinctly rounded corners, and is parallel to the plane of three of the nuclei. An optical section of the protoplast, cut parallel to any of the four faces in the plane of three of the nuclei, is an equilateral triangle with rounded corners and a nucleus near each corner. The cytoplasm extending between any two of the nuclei now appears quite fibrillar and closely resembles a spindle (fig. 9). A careful examination of the preparations, however, shows that these spindle-like regions are modifications in the form of the cytoplasm, and that the spindle-like appearance is caused by the formation of elongated vacuoles, causing the material on

either side of each vacuole to become stretched, and therefore fibrous. That these are not real spindle fibers but normal elements of the cytoplasm is shown by their staining reaction. When safranin and gentian violet are used, the chromatin and cytoplasm stain red and the pollen-mother-cell wall violet. If these regions extending between the nuclei were composed of true spindle fibers like those of the heterotypic and homoeotypic spindles, the spindle fibers should stain violet. No change in the balance of the two stains was effective, however, in securing a differential staining reaction between the peripheral region of the cytoplasm and the regions extending between the nuclei. The writer concludes, therefore, that the regions extending between any two of the nuclei do not have true spindle fibers, but are modifications in the form of the cytoplasm. Kirkwood's (11) figure 31 shows a very similar condition in *Micrampelis* at this stage, although he does not describe it.

Four equidistant conical projections now appear on the inner surface of the special callose wall, one at the center of each of the four faces of the tetrahedral mother cell, the center of each face being equidistant from each of three nuclei which are in a plane parallel with that face. Each projection is connected with the other projections by ridges on the inner surface of the mother-cell wall, each of which is perpendicular to, and cuts through the center of, the edge of the tetrahedral protoplast which it crosses. Consequently there are six ridges, the intersection of any three of which is a conical projection. These projections advance centripetally more rapidly than the connecting ridges, each remaining equidistant from the three nuclei at the corners of the face on which it originated, and moving toward the fourth nucleus. Eventually the four ridges on the inner surface of the mother wall fuse at the center of the cell, cutting the mother cell into four uninucleated protoplasmic masses, the microspores (fig. 12).

Reference has already been made to Nägeli's (15) study of the formation of microspores in *C. Pepo*. He considered constriction as occurring by a process of cell-plate formation. As Nägeli worked with living material, it is not surprising that he mistook the ingrowing extensions of the mother-cell wall for cell plates, since, as shown by the writer's figures 10 and 12, the septa are at first extremely thin. It seems very unlikely that Nägeli saw the cell plates, for, as stated above, they are very transitory and the spindles are so small that the cell plates would be very difficult to observe in living material.

The thin septa which effect the quadripartition of the protoplast are centripetal extensions of the special callose wall and are initiated, as has been shown, by thickenings on the inner surface of this wall. The ridges (fig. 9) at first progress centripetally by the deposition of callose on their inner edges, and up to this point their centripetal advance is identical with that recently described in *Melilotus alba* by the writer (3). A marked departure now occurs, however. In *Melilotus* it was found that furrowing

was effected by the fusion of small vacuoles at the regions of cytoplasm midway between each pair of nuclei, forming large vacuoles which eventually were separated only by narrow strands of cytoplasm. Incoming ridges then appeared on the special wall, advancing centripetally by the deposition of callose on their edges, and severing the narrow strands of cytoplasm which separated the vacuoles, thus making the furrows complete. These septa continued to grow toward the center of the cell until they fused, thus delimiting the four microspores. In *Cucurbita maxima*, however, the writer found that, when the ingrowing septa have advanced a short distance, thickenings begin to appear on strands of the cytoplasm midway between each pair of nuclei. Figure 11 shows a portion of the mother cell in which a septum is advancing centripetally. The right of the figure shows the beginning of thickenings on strands of the cytoplasm. In passing to the left the thickenings become more compact and then wider in extent, finally fusing together side by side and then being met by, and incorporated into, the ingrowing septum. It is evident, therefore, that the cell plates, which are transitory, play no part whatever in the quadripartition of the protoplast.

After the septa have come together at the center of the tetrad (fig. 12), the special wall, which now surrounds each young microspore, becomes much thicker, evidently by the secretion of callose from the individual protoplasts (fig. 13). The line at which any two of the individual special walls adjoin appears in living material as a seam. The four seams come together at the center of the tetrad and in optical view appear as a triradiate seam.

When the special wall attains its maximum thickness (fig. 13), the exine begins to form, and the spines, which are so conspicuous on the mature pollen grain, begin to appear. The special wall now begins to break down, first at the outer edges of the wall closest to the young pollen grains. The pollen grains then escape from the surrounding callose wall.

A careful study of serial sections shows that the inner margin of the septum is not a straight edge, but is wavy, as was also found by Wanda K. Farr (9) in *Cobaea scandens alba*. A somewhat similar condition was found by C. H. Farr (8) who reports that the inner margin of the partition wall in *Nelumbo lutea* is perforated. The writer's figure 10 shows evidence that the margin is wavy. The septum at the left of the figure shows a portion of the wall which is apparently unconnected with the wall on either side. The next serial section, however, shows the septum continuous from the special wall to the center of the cell. It is easy to understand how an apparently isolated sector of the wall as here shown would lead a superficial observer to regard it as very good evidence that the partition wall is formed by the cell-plate method. Figure 10 shows that the four septa do not advance equally rapidly. The projection at the top of the figure has just begun to advance whereas the other two septa shown have already met at the center.

When the homoeotypic spindles occur parallel to each other, the four nuclei become arranged equidistant in a plane. The spherical protoplast gradually assumes the shape of a half cube with rounded corners, the two isodiametric faces of which are slightly convex; the other four faces are rectangular, with the long side twice the length of the short one. These four faces are more distinctly convex than are the other two.

Thickenings appear on the inner surface of the special callose wall at the center of each of the six faces. The thickenings on the rectangular faces are wedge-shaped with the two lateral faces concave. An optical section of the mother cell in the plane of the four nuclei shows four centripetal projections of the callose wall, one midway between each pair of nuclei. These projections appear triangular, two of the sides being concave and toward the center of the cell tapering to narrow lines. The thickenings on the flat faces of the callose wall are different from those on the other four. The thickening at the center of the inside of each flat face appears at an early stage as a pyramid with four lateral faces, each of which is in outline an isosceles triangle with the equal sides concave and with the center of the face drawn toward the center of the pyramid. The mother cell at a slightly later stage appears as a half cubical body with two ridges, which in cross section are triangular and which extend around the cell at right angles to each other midway between the nuclei. Each ridge bisects all the faces of the cell over which it passes. These ridges are distinctly arched beneath the edges of the cell, and a deeper projection of the ridge occurs at the center of each of the square faces. This projection is pyramidal, with four concave sides. The ridges continue to advance until they fuse, cutting the mother cell into four uninucleated protoplasmic masses. The details of the formation of the septa by thickenings on strands of the cytoplasm are as described above for the tetrahedral mother cell. The four microspores with the surrounding callose walls may be compared to four equidistant slightly ellipsoidal bodies surrounded by a mass of gelatin, the ellipsoids and gelatin together forming a half cube with slightly rounded corners and with slightly convex faces.

#### DISCUSSION

It has been noted that in *Cucurbita maxima* cell plates appear on both the heterotypic and the homoeotypic spindles; that these cell plates disappear soon after being formed and, therefore, take no part whatever in the quadripartition of the pollen mother cell. Lubimenko and Maige (12) have unquestionably seen cell plates which are very similar to those found in *Cucurbita maxima*. That investigators have not more often found these cell plates, especially on the homoeotypic spindles, is probably because of the ephemeral nature of the plates. The writer, after studying many hundreds of sections, had come to the conclusion that cell plates do not occur in *Cucurbita*. The study of a still larger number of sections, however, produced several slides, with sections from as many different flowers, which

gave unmistakable evidence of cell plates. Many more sections were found showing cell plates on the heterotypic spindle than on the homoeotypic. It seems very probable that in some cases in which quadripartition has been reported by a process of cell-plate formation, the workers have seen cell plates such as shown for *Cucurbita* and, not noting their subsequent disappearance, have assumed that they took part in the division of the mother cell. Lubimenko and Maige (12) state that after the homoeotypic spindles and cell plates are resorbed new spindle fibers are formed by the cytoplasm, and new cell plates, which effect the quadripartition of the mother cell, appear on these fibers. It is very probable that these two workers found a situation similar to that which the writer has found in *Cucurbita*. What they consider new spindles arising from the cytoplasm after the homoeotypic spindles have been resorbed, the writer finds in *Cucurbita* to be modifications in the form of the cytoplasm, as shown by the staining reaction. Moreover, what they regard as cell plates appearing on these new spindles are doubtless thin partition walls or sectors of them.

A question which naturally suggests itself is why the cell plates are formed and then disappear, apparently without serving any function. Timberlake (18) has suggested that the reason for the failure of the cell plates to function in the division of the protoplast is that no peripheral spindle fibers are formed between the central spindle and the plasma membrane, and therefore the cell plate can not extend to the plasma membrane. This explanation seems plausible but gives no suggestion as to why peripheral fibers fail to develop. The writer, however, has no further explanation to offer.

#### CONCLUSIONS

1. During early diakinesis a thick, homogeneous callose wall is formed around the protoplast of the pollen mother cell.
2. A dense region of cytoplasm, the perinuclear zone, is formed around the nucleus. As the nuclear membrane disappears, fibers, originating from the nuclear cavity, form several cones which constitute the multipolar spindle.
3. The haploid number of chromosomes is 20; the diploid, 40.
4. Definite but transitory cell plates are formed on the heterotypic and homoeotypic spindles. These cell plates take no part whatever in the division of the pollen mother cell.
5. The callose mother cell wall shows distinct lamina. After the heterotypic division is completed a "special wall" is formed just inside the original mother cell wall. The special wall is composed of denser, more refractive callose than the original wall.
6. Quadripartition of the pollen mother cell is effected by partition walls which originate on the inner surface of the special wall and at first grow centripetally by the deposition of callose on their inner edges. Thickenings

These thickenings condense, fuse side by side, and then become incorporated into the ingrowing septum. The septa unite at the center of the cell, dividing the mother cell into four uninucleated protoplasmic masses, the microspores.

The writer is indebted to Dr. J. N. Martin for helpful suggestions during the course of the investigation and to Ellen Wright Castetter for considerable help in working out the relation of the partition walls to the division of the pollen mother cell in the formation of the microspores.

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## EXPLANATION OF PLATES

## PLATE I

FIG. 1. Pollen mother cell, showing multipolar spindle surrounded by perinuclear zone. Protoplast surrounded by thick callose wall.  $\times 1150$ .

FIG. 2. Heterotypic spindle, showing univalent chromosomes just after they have pulled apart.  $\times 2300$ .

FIG. 3. Polar view of spindle at equatorial-plate stage, showing the 20 bivalent chromosomes.  $\times 2600$ .

FIG. 4. Polar view of spindle at equatorial-plate stage from root-tip cell, showing 40 somatic chromosomes.  $\times 2300$ .

FIG. 5. Heterotypic spindle at telophase. Chromosomes anastomosing. A distinct cell plate present at the equator.  $\times 2300$ .

FIG. 6. Daughter nucleus preparatory to homoeotypic division. Both members of each of the univalent chromosomes, and the nucleolus, are seen.  $\times 2300$ .

FIG. 7. Telophase of homoeotypic division, the spindles parallel to each other. The ephemeral cell plates are distinct.  $\times 2300$ .

## PLATE II

FIG. 8. Homoeotypic spindles at right angles. Spindle at right, cut at equatorial region, shows 20 univalent chromosomes. (This figure logically precedes fig. 7.)  $\times 2300$ .

FIG. 9. Pollen mother cell, showing vacuolated fibrous cytoplasm at equators of former spindles. Three of the four incoming ridges of the callose wall seen.  $\times 950$ .

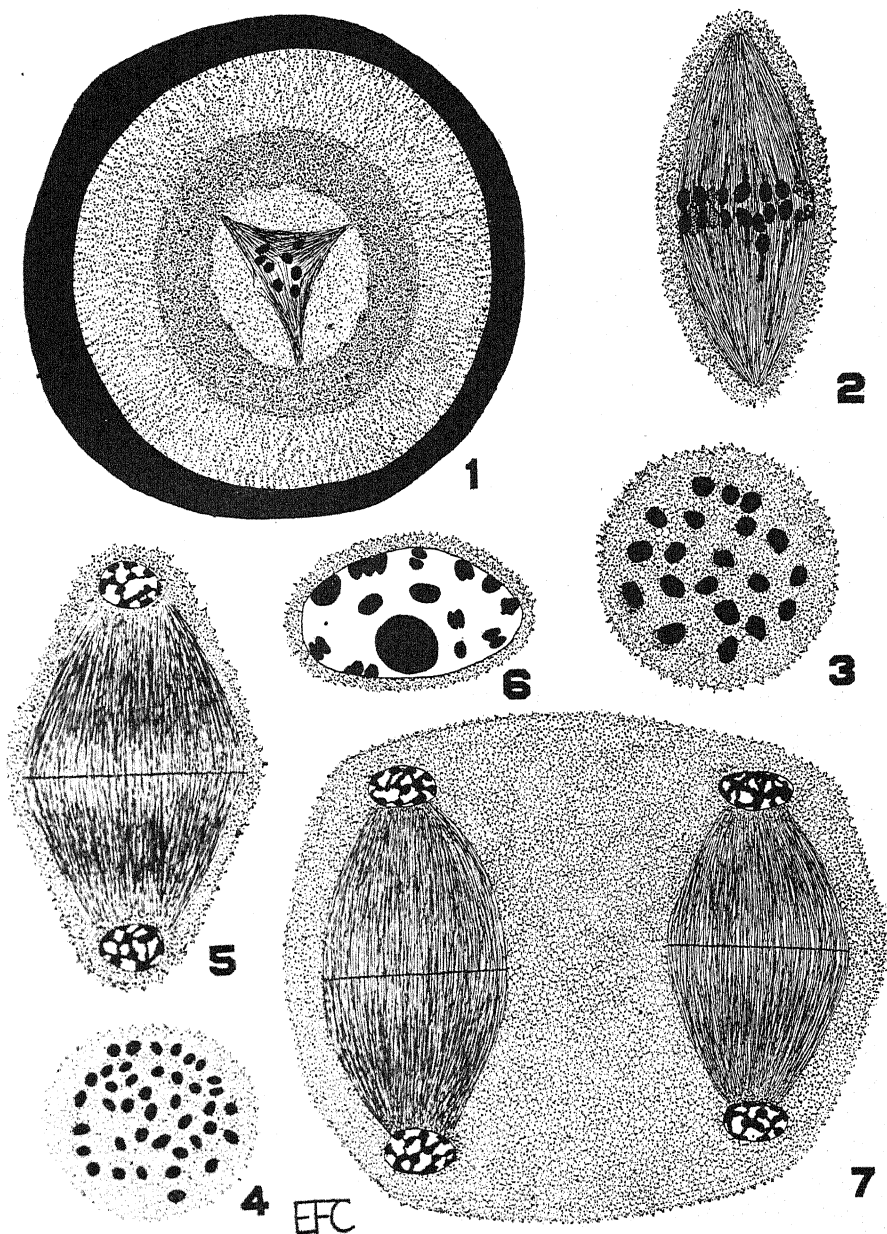
FIG. 10. Pollen mother cell in which the septa have advanced centripetally and have met at the center. This view gives evidence of the wavy margins of the septa.  $\times 950$ .

FIG. 11. Sector of a pollen mother cell showing an incoming septum. Stages in thickening of strands of cytoplasm are seen.  $\times 2300$ .

FIG. 12. Mother cell in which the septa have fused in the center, forming the four microscopes.  $\times 850$ .

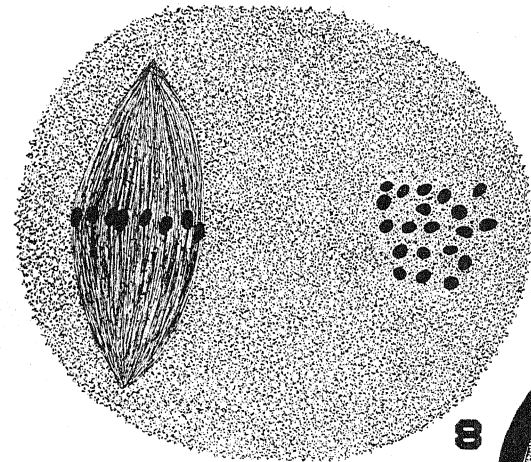
FIG. 13. The callose septa have thickened considerably. This stage just precedes the formation of the exine and intine.  $\times 900$ .



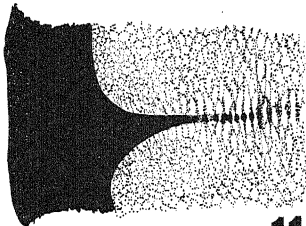




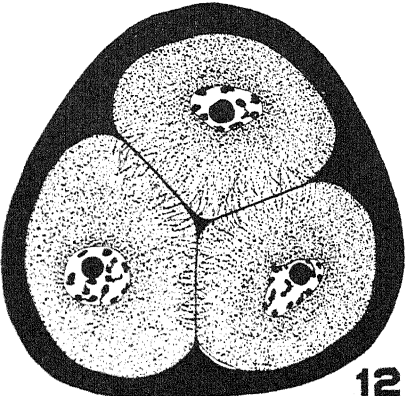




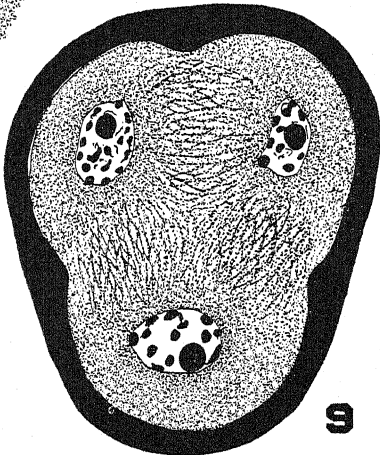
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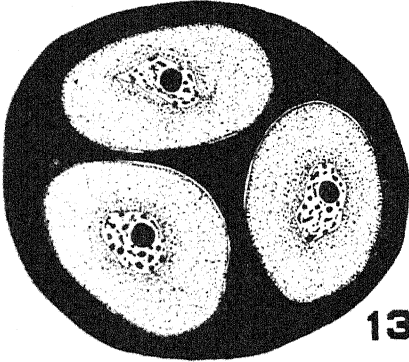
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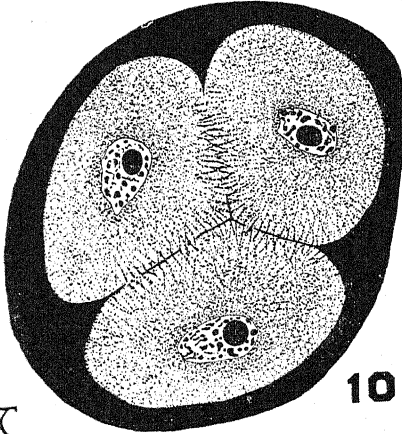


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## THE GENERA FLAMMULA AND PAXILLUS AND THE STATUS OF THE AMERICAN SPECIES<sup>1</sup>

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### FLAMMULA (FR.) QUÉL.

The Agarics of the ocher-spored group have had my attention during many years. During this period, I have from time to time put on record such data as I was able to gather concerning the occurrence and distribution of the American species of *Cortinarius* (11) and *Inocybe* (13). But field studies were continuously made also of the other genera of the group. However, it became increasingly difficult to identify with any feeling of certainty the collections of the genus *Flammula* that were picked up. During recent years, especially since the appearance of Murrill's (14) compilation of the descriptions of American species, special attention was directed to this genus. It soon became evident that the genus *Flammula* was being used as a dumping-ground for species that did not seem to fit elsewhere—in other words, the generic limitations of this genus were no longer respected.

It will be necessary then to review the conceptions of the genus held by those who first limited the group and gave it scientific standing. Persoon (18), as well as others of those early days, scattered such species as we can still roughly recognize among several sections of the old genus *Agaricus*. Fries (5), under his tribe XXV, gives the word "*Flammula*" its first characterization as follows:

Veil marginal, fibrillose, very fugacious, not glutinous. Stipe at first stuffed, then for the most part hollow, not bulbous, firm, fibrillose (not appressed-scaly from a transversely ruptured veil) homogeneous with the pileus. Pileus fleshy, convex-expanded, not striate, glabrous (very rarely with superficial scales and these not innate), dry or viscid. Flesh not thick, but firm. Gills not emarginate, variable.

In the *Monographia* (6), which is far and away the most carefully done and most useful of any of the works of Fries dealing with Agarics, we find the tribe "*Flammula*" distinguished as follows (1: 348):

The *Flammulae* are distinguished among the ocher-spored Agarics by the fleshy-fibrous stipe, by the filamentous or obliterated veil, by the fleshy pileus, and by the acute, adnate or decurrent lamellae.

He adds further:

With reference to their natural affinity, it is clear from their bright-colored spores that they are nearest to the *Pholiotae*; from the *Hebelomata* and *Inocybes* they are far removed by their very nature. They form a natural group, although they represent four quite distinct types and the species of the first section differ so much from each other that one

<sup>1</sup> Papers from the Department of Botany of the University of Michigan, no. 224.

could consider each single one as a type peculiar to itself. In most of the species the spores are pure ferruginous, but in *A. carbonarius* [= *Flammula*] they are fusco-ferruginous, and in *A. sapineus* and its relatives they are fulvo-ochraceous. Some species are terrestrial, some lignicolous, and some thrive in either situation; for the most part they occur late in the season. In most the taste is insipid or bitter and they are therefore not edible.

In *Hymenomycetes Europaei* (7, p. 244), we find the same conception of the generic limitations as that just quoted, except for the addition of the emphatic statement that "the margin of the pileus is at first involute."

The other mycologists of the period covered by Fries' writings, as well as most of those who have since written Agaric manuals, have kept close to the meaning of the genus as defined in the quotation from *Monographia*. For example, Quélet (19), who officially raised the group to the rank of a genus, merely by writing "genre" before the word *Flammula*, characterizes it thus: "Stipe charnu, lamelles adnées ou décurrentes," and places it between *Hebeloma* and *Naucoria*. Saccardo (22) cited the *Systema* of Fries, but copied the generic description from *Hymenomycetes Europaei*. Of recent works, in which the genus is kept intact, Ricken's *Blätterpilze* (21) follows the Friesian conception and retains the species. Ricken places the genus between *Pholiota* and *Naucoria*, but remarks that the genus *Hypholoma*, except for its purple-brown spores, is very similar. Rea (20), breaking away from the traditional use of grouping the genera of similar spore color together, has placed the genus *Flammula* in his group of decurrent-gilled Agarics, along with *Clitocybe*, *Hygrophorus*, *Clitopilus*, and *Gomphidius*. This arrangement, with the phylogeny to be assumed as accompanying it, is of doubtful validity. It has already been criticized by Buller (3). The idea is not new. To quote W. G. Smith (23):

Fries says the natural affinity of *Flammula* is with *Pholiota*, but I consider all true *Flammulas* should correspond with *Clitocybe* and *Clitopilus*. I suspect some of the species of *Flammula* that approach *Pholiota* in structure might with propriety be removed to that subgenus and *Flammula* proper be restricted to species with decurrent gills.

There are, however, insurmountable difficulties in the use of the decurrent-gill character alone, since many species, with undoubtedly complete *Flammula* characters otherwise, have adnate gills. At the same time, Rea retains the fundamental Friesian characters of the genus, hoping to strengthen his position by limiting the adnate-gilled species to those with a decurrent tooth. To my mind, this can not be done without establishing other genera.

Turning now to other conceptions of the genus, we are met by the name *Gymnopilus*, proposed in 1879 by P. Karsten (9) for a portion of the old genus, and *Gymnocybe* for another portion. The former of these was selected by Murrill in his treatment of the genus in the *North American Flora* (14). The generic conception presented here is the same old Friesian conception. Quite a number of the species, however, which Murrill included, do not submit to the requirements laid down in his own generic description. These, as will be seen in the section of the present paper dealing with excluded species, had to be removed.

Fayod (4) divided the species among the genera "*Flammula* Fr.," "*Flammopsis*," and "*Ryssospora* gen. nov." His own knowledge of the species of this group was undoubtedly scanty, and little use can be made of his statements about them. Hennings (8) reversed the method used by the preceding authors and "lumped" a number of Friesian genera, including most of the species of *Flammula*, under the genus *Naucoria*.

As far as I can see, no changes have been made in the Friesian conception of the generic limitations since the appearance of *Hymenomycetes Europaei*. The first paragraph from Fries' *Monographia* quoted above is a clear-cut statement of the characters of this genus. The further character of the involute young margin of the pileus has not been, as a rule, noted by authors of American species, so that it must, of necessity, be neglected for the present. A number of the species are now known to be characterized by cystidia in the hymenium, a fact, as we shall see below, that is of considerable diagnostic importance. Where changes have been attempted, authors contented themselves with taking the old facts, mainly established by Fries, and devising different arrangements of them. It would have furthered our knowledge of the genus much more if their efforts had centered on the development and anatomy of the known European species.

Ricken (21), following in the footsteps of Bresadola (1), grasped the importance of getting new facts as well as the need for the study of living plants, and his records of the spores and the cystidia are of inestimable value to the American student. Much still needs to be done along this line in Europe, but a good start has been made. In this connection, it is very much to be regretted that Rea (20) has not given us his own critical account of all the microscopic characters of the British plants of this genus. Some dozen British species are here excluded because of the lack of such data.

The outstanding surprise of my study of the types of the species described in America was the fact that about half of the valid species had distinctly recognizable cystidia in the hymenium. This reduces the difficulty of recognizing the species at once to a remarkable degree. The presence or absence of cystidia, their character, shape, and size, are quickly determinative, where synonyms are open to question, or where species are alike. I had long known, from my own studies in the field, that a considerable number of species must possess cystidia, but the omission of this character in so many of Peck's and Murrill's descriptions made me wary and uncertain.

It is further surprising that, with the exception of Ricken, the cystidia in this genus have been rarely mentioned. Even Patouillard (16, 17), who paid special attention to the hymenial structure of most of the genera, makes no mention of them in this genus.

The spores of many species are too similar in size to be dependable, although some species are clearly to be kept separate on spore characters. Fayod (4) and others have attempted to emphasize the germ pores present

in the spores of a number of species, and eventually this character may prove of some value in the study of the relationships of the species of *Flammula*.

Since most species of *Flammula* are lignicolous, it is of considerable interest to know to what extent their occurrence is limited by the nature of the wood on which they occur. Although saprophytes, we know that for many basidiomycetes a coniferous substratum on the one hand, and wood from frondose trees on the other, are frequently limiting factors. In so far as sufficiently reliable data are at hand, I have attempted to emphasize this feature in the following synopsis. Fries himself emphasized it. The "Sapineae," occurring on coniferous wood, are a natural group, not only in this respect but apparently also in the fact that these species lack cystidia and have a non-viscid pileus.

One hundred and five names, applied to species of *Flammula*, are referred to in this paper. About half of these have been placed in the excluded list. There remain fifty-two species with a more or less excellent claim to validity. These include those American and European species about which we have fairly definite information.

For the present, and until the American species are better known, it is futile to attempt any detailed discussion of relationships within the genus. The subdivisions made by Fries are at present convenient enough as receptacles for the added American species, and much collecting and careful microscopic work is necessary before the knowledge of our species is at all adequate. This paper has been prepared in the belief that it will establish an entirely new starting point for the study of American species of *Flammula*, and as many data as space permitted have been included to promote such a study.

I am under special obligation to the authorities of the New York Botanical Garden for permission to examine the types of Dr. Murrill's species; to Dr. House at Albany for free access to Dr. Peck's species; and to the Department of Plant Pathology of the New York State College of Agriculture for opportunity to study the collections of *Flammulae* in the Atkinson Herbarium.

Practically all American type specimens have been carefully gone over. A few could not be found or are no longer in existence. It is not likely that the number of American species is as great as in some of the related genera of the *Ochrospora*e, and great care should be exercised in describing new ones because of the close similarity of species from the related genera, such as *Cortinarius*, *Pholiota*, and *Naucoria*.

### Synopsis of the Species of *Flammula* of the North Temperate Zone

1. Cystidia present, mostly abundant.....2.
1. Cystidia lacking (frequently sterile cells occur on the edges of the gills; these may be cystidia-like but are shorter).....22.
2. Spores not over 10  $\mu$  long.....3.

2. Spores 12-16 x 6-7  $\mu$ ; odor and taste mild; pileus 3-5 cm. broad; viscid, glabrous, isabelline or with brownish disk; gills broad, decurrent by lines; stem 5-8 cm. x 7-10 mm.; in coniferous forests, on or near coniferous wood (Europe).  
*F. mixta* Fr.-Ricken.
3. Pileus viscid, glutinous, or sub-viscid, pellicle separable . . . . . 4.
3. Pileus moist or dry, not viscid . . . . . 19.
4. Pileus glabrous . . . . . 5.
4. Pileus at first with superficial scales from the universal veil, at least on the margin . . 14.
5. Plants caespitose or subcaespitose or densely gregarious . . . . . 6.
5. Plants solitary or loosely gregarious . . . . . 11.
6. Spores 7-8.5 or 7-10  $\mu$  long; odor noticeable (except in *F. fibrillosipes*) . . . . . 7.
6. Spores 5-7 (7.5)  $\mu$  long; odor none or slight . . . . . 9.
7. Odor penetrating, rather strong, especially when the plant is crushed or during drying . . . . . 8.
7. Odor none; taste subacid; pileus reddish-brown, darker on disk, at first appendiculate on margin; stem densely fibrillose, solid; cortina copious, persistent; spores 7-8.5 x 3.5-4.5  $\mu$ ; cystidia few to scattered (Alabama) . . . . . *F. fibrillosipes* Murrill.
8. Spores 7.5-10 x 4.5-6  $\mu$ ; gills broad and almost subdistant, at first yellowish; pileus brownish-yellow, umbo darker; densely caespitose, cystidia rather abundant; on pine wood or clearings in pine wood . . . . . *F. condensata* Pk.
8. Spores 6-8(8.5) x 4-4.5  $\mu$ ; gills medium broad, close; pileus yellow or yellowish with tawny centre, obtuse pellicle subseparable or adnate; flesh of pileus and stem pale yellow; cystidia abundant; cortina evanescent; on or about coniferous wood.  
*F. graveolens* Pk.
9. Stem slender, 2-4 mm. thick; pileus 2-4 (5) cm. broad; gills at first pallid . . . . . 10.
9. Stem 3-8 (10) mm. thick, 3-7 cm. long, fibrillose, stuffed-hollow; pileus 2-5 cm. broad, yellow shades predominating, disk darker; margin of pileus, *young gills and flesh of pileus citrine-yellow*; cortina evident, citrine; gills adnate, decurrent by tooth; in coniferous and frondose (?) woods. Spores 5-6.5(7) x 3.5-4  $\mu$  (Europe and America).  
*F. spumosa* Fr.-Ricken.  
*F. subflavida* Murrill.  
*F. piceina* Murrill.
10. Growing on charred wood or burnt-over ground, densely gregarious or subcaespitose; gills rather broad, close to crowded; pileus brownish-incarnate to dull reddish-brown; spores 6-7 x 3-4  $\mu$  (Europe and America).  
*F. carbonaria* Fr. (sense of Ricken, Patouillard, and Rea).
10. Growing in swampy woods, especially under alders, caespitose; gills adnate; stem subcartilaginous; pileus sordid yellowish-rufescent; spores 6-7 x 4-4.5  $\mu$  . . . . . *F. squalida* Pk.
11. Pileus 5-9 cm. broad; stem 10-15 mm. thick . . . . . 12.
11. Pileus not over 5 cm. broad . . . . . 13.
12. Gills near maturity rusty, tinged with gray-olivaceous; stem rooting-attenuate, fibrillose-streaked, concolor; pileus subtestaceous; spores 8-9 x 4-5  $\mu$ ; on and about old logs (Europe) . . . . . *F. fusa* Fr.-Rick.
12. Gills cinnamon-fulvous at maturity; stem equal or incrassate below, whitish, with yellow mycelium at base; pileus rusty-fulvous, pallid on margin; spores 6-7 x 3.5-4  $\mu$ ; on log of frondose tree (western United States) . . . . . *F. lata* Murrill.
13. Hymenium with metalloid cysts; pileus 2-3.5 cm. broad, yellowish-red; stem hollow, minutely floccose-scaly; spores 6-8 x 4-4.5(5)  $\mu$ ; cystidia subabundant, scattered or few, on and near edges of gills; on burnt ground in the open, or on *débris* in woods (*F. ascophora* Pk.; *F. pusilla* Pk.) . . . . . *F. highlandensis* Pk.
13. Hymenium without such cysts; pileus 3-5 cm. broad, bay color or with yellow tints; gills broad, at first citrine; stem fibrillose, equal, 10-15 mm. thick; spores 6.5-7.5



- x 3.5-4  $\mu$ ; cystidia scattered, on and near edges of gills; on a railroad tie (western United States)..... *F. vialis* Murrill.
14. Pileus 5-12 cm. broad; stem whitish..... 15.
14. Pileus up to 6 cm., rarely 7 cm. broad..... 16.
15. Pileus "zinc-orange" (Ridg.), darker or tawny-fulvous on disk, glutinous, stem loosely fibrillose; spores 5-7 x 3-4  $\mu$ ; on or about decayed logs, *débris*, etc. (Europe and America)..... *F. lubrica* Fr.
15. Pileus pale buff to grayish, glutinous, viscid, or subviscid; gills adnate-subdecurrent or with tooth; cystidia abundant, 50 x 12-14  $\mu$ , stout.
- (a) Pileus 5-12 cm. broad; on white birch; spores 6-7 x 4-5  $\mu$ , subreniform.  
*F. betulina* Pk.
- (b) Pileus 2-7 cm. broad; on much decayed wood *débris* or leaf mold; spores 5-6.5 x 3-4  $\mu$ , short oblong..... *F. lenta* Fr.
16. Plants more or less caespitose; spores 6-7.5 x 3.5-4  $\mu$ ..... 17.
16. Plants solitary or scattered on sticks and fallen branches; spores 5-6.5 x 3-4  $\mu$ ; pileus yellowish to dull orange on disk, greenish-olivaceous on margin, decorated with creamy scales, 3-7 cm. broad; gills at length olivaceous-purplish-gray, adnate or spuriously subdecurrent; stem slender, 3-5 mm. thick, scaly; on sticks or fallen branches (eastern United States)..... *F. polychroa* Berk.
17. Pileus pale cream-color or pale ochraceous, with olive hues, flesh whitish. (See also *F. betulina* Pk.)..... 18.
17. Pileus sordid-tawny, unicolorous, darker appressed scales on margin; flesh grayish-white; cystidia lanceolate, 60-75  $\mu$  long, slender with wavy outlines; about the bases of trunks, etc..... *F. subfulva* Pk.
18. Stem slender, 3-6 mm. thick, creamy-white, fibrillose-scaly; scales on disk or umbo of pileus "bay" (Ridg.) or chestnut; cystidia very abundant, 60-75  $\mu$  long; on fallen branches (Western United States)..... *F. decorata* Murrill.
18. Stem 4-10 mm. thick, base rusty-reddish when bruised; scales of pileus evanescent; cystidia scattered, 45-50  $\mu$  long; about the bases of trunks and stumps (Europe and America)..... *F. gummosa* Fr.
19. Pileus 5-10 cm. broad; stem 7-10 mm. thick, densely caespitose..... 20.
19. Pileus 2-5(6) cm. broad; subcespitose..... 21.
20. Spores 6-7 x 3-4  $\mu$ ; cystidia 60-75 x 12-17  $\mu$ ; pileus not viscid, straw-yellow with rusty-brown disk, margin with shreds of veil; gills broad; odor penetrating, strong; taste bitter; on stumps (Europe and America!) (*F. alnicola* Ricken)  
*F. amara* Bull.-Ricken.
20. Spores 8-9 x 4-5  $\mu$ ; cystidia 36-40 x 8-9  $\mu$ ; pileus not viscid, sulfur-yellow, at first with pale appressed scales on surface or margin; gills rather narrow; odor none or earthy; on and about trunks (Europe and America)..... *F. flavida* Fr.-Ricken.
21. Pileus hygrophanous, dark umber when moist, fading; gills subdistant to distant; stem tough, 4-6 cm. long, 4-6 mm. thick; spores 7-9 x 4-5  $\mu$ ; on rotten logs (Alabama)..... *F. unicolor* Murrill.
21. Pileus not hygrophanous, sulfur-yellow, disk reddish-orange; cortina copious; stem 2-5 cm. long, 2-4 mm. thick; cystidia abundant, with wavy outline; spores 6.5 x 4-5  $\mu$  (western United States)..... *F. velata* Pk.
22. Growing on wood of coniferous trees, logs, stumps, decayed remnants, etc.; pileus dry or moist, not viscid..... 23.
22. Growing on wood of frondose trees, rarely on the ground; pileus viscid, subviscid, or dry..... 34.
23. Stems elongated when normal, 5-10(12) cm. long..... 24.
23. Stems shorter, varying, 2-5 cm. or 5-7 cm. long..... 28.
24. Stems slender, 2-5 mm. thick; pileus glabrous; plants subcespitose..... 25.
24. Stems rather stout, 6-12 (15-20) mm. thick..... 26.

25. Pileus red-orange-incarnate, deeper color on disk, 3-5(7) cm. broad, margin whitish-silky; stem stuffed-hollow, fibrillose to subscaly; taste bitterish; spores 6-7 x 3-3.5  $\mu$  (Europe and America)..... *F. astragalina* Fr.
25. Pileus alutaceous-honey color, 4-8(10) cm. broad; cortina very evanescent; stem fistulose, pallid, at length testaceous or fuscous, appressed-fibrillose; gills narrow, at length grayish-olivaceous; spores 6-7.5(8) x 4-4.5  $\mu$  (Kauff.) sordid rusty (Europe and America)..... *F. inopoda* Fr.
26. Pileus some shade of yellow; plants cespitose.....27.
26. Pileus dull green, 4-8(10) cm. broad; stem and pileus at first with pulverulent bluish-green bloom; gills greenish; taste bitter; spores 5-7 x 3.5-4  $\mu$  (western United States)..... *F. subviridis* Murrill.
27. Pileus 10-15 cm. broad, pale yellow, deeper-colored in age, subfibrillose; stem 7-10 cm. x 12-20(25) mm., solid, subequal, concolor; spores 9-10 x 5-6  $\mu$ ... *F. magna* Pk.
27. Pileus 4-10 cm. broad, yellowish-brown, minutely scaly; stem radicating, streaked; spores 6-7.5 x 4-5  $\mu$  (Alabama)..... *F. underwoodii* Pk.
28. Pileus more or less floccose-scaly with small scales, or floccose-granulose.....29.
28. Pileus glabrous; plants subcespitosae; base of stem white-villose; gills yellow or golden, then fulvous to rusty.....31.
29. Stem 3-8 mm. thick.....30.
29. Stem slender, Naucoria-like, 2-3 mm., lacerate-subscaly; pileus 3-5 cm., rusty-fulvous; spores 6-7 x 3-4  $\mu$  (Schroeter) (Europe)..... *F. limulata* Fr.
30. Pileus slightly viscid when moist, soon dry, more or less yellow, with pink or purplish shades especially on disk, 2.5-5 cm. broad; gills broadly adnate, becoming bright tawny-ochraceous, spores 6-7.5(8) x 4-5  $\mu$  (syn. *F. pulchrifolia* Pk.)... *F. braendlei* Pk.
30. Pileus always dry, golden-fulvous, tawny or rusty yellow, often diffringed, 3-8 cm. broad; stem persistently stuffed, equal, sometimes eccentric, fibrillose to subscaly; gills rather broad, adnate, becoming fulvous-cinnamon; spores 7-8(9) x 4-5  $\mu$  (Europe and America) (syn. *F. eccentrica* Pk.)..... *F. sapinea* Fr.
31. Cortina none; flesh of pileus colored; stems slender.....32.
31. Cortina evanescent or copious; flesh of pileus white to pallid; stems rather stout, tapering upward.....33.
32. Stem umber-colored, at first everywhere covered by whitish pulverulence, fistulose, slender, 2-5 mm. thick; gills very narrow; pileus bay-brown, 2-4 cm. broad; spores 8-10 x 5-6  $\mu$  (Schroeter) (Europe)..... *F. picrea* Fr.
32. Stem fulvous, then ferruginous, naked, 4-7 mm. thick; gills rather broad; pileus 3-8 cm. broad, very glabrous, golden-yellow to orange-fulvous, spores 8-9 x 4.5-5.5(6)  $\mu$  (Kauff.) (Europe and America)..... *F. liquiritiae* Fr.
33. Gills becoming fulvous-spotted in age, at first whitish; pileus dry, yellow-fulvous, fading in age, 5-8 cm. broad; spores subventricose, 6-7.5 x 3.5-4.5  $\mu$  (Europe and America)..... *F. penetrans* Fr.
33. Gills not spotted in age, at first yellow; pileus moist, at first cinnamon-fulvous, becoming orange-fulvous, 5-8 cm. broad; stem white-cortinate; spores 7-9(10) x 4-5(6)  $\mu$  (Ricken) (Europe)..... *F. hybrida* Fr.
34. Pileus viscid or with subviscid pellicle, glabrous or subfibrillose.....35.
34. Pileus not viscid.....42.
35. Spores not with rufous tints, usually smaller, ellipsoid.....36.
35. Spores in mass "cinnamon-rufous" (Ridg.), 9-12 x 4-5(5.5)  $\mu$ , fusiform; pileus soon dry, "apricot-yellow"; gills yellowish-cinnamon, then "saya-brown"; stem rigid, 4-8 mm. thick, cespitose; base of trunks (Europe and America)..... *F. comissans* Fr.
36. Stems slender, 3-7 mm. thick.....37.
36. Stems stouter, 5-10(15) mm. thick.....40.
37. Plants cespitose to subcespitosae.....38.
37. Plants solitary or few; pileus dark rich brown, 3-5 cm. broad; flesh dark yellow;

- stem 4-5 cm. long, yellowish-brown; taste mild; spores  $6.5-8.5 \times 3.5-4 \mu$ ; on the ground (!) in pine woods (Alabama) ..... *F. castanea* Murrill.
38. Pileus not hygrophanous nor sulfur-yellow (dry) ..... 39.
38. Pileus hygrophanous, distinctly viscid, umbonate, watery-yellow (moist), sulfur-yellow (dry), 2-6 cm. broad; gills narrow, arcuate; spores  $8-10 \times 4-5 \mu$ ; under apple trees (*F. sulphurea* Pk. non Massee) ..... *F. malicola* nom. nov.
39. Pileus yellowish, disk fibrillose and tinged pale rose-brown, soon dry, 2-3 cm. broad; stem glabrous above, shaggy-fibrillose below, sub-concolor; spores  $5-6 \times 3.5 \mu$ ; roadside (western United States) ..... *F. ornatula* Murrill.
39. Pileus bright yellowish-brown, reddish-brown on disk, distinctly viscid; gills subdistant; stem whitish, hollow, subfibrillose, 5-6 cm. long; spores  $7-8 \times 3.5-4 \mu$ ; on clay banks! (Alabama) ..... *F. alabamensis* Murrill.
40. Spores  $9-10(11) \times 5-6 \mu$ ; pileus soon dry ..... 41.
40. Spores  $6-7 \times 3-4 \mu$ ; pileus pale ochraceous, mixed with buff and olive hues, sometimes cortinate-scaly on margin, glutinous; stem 4-6 cm. long, pallid above, at length reddish-umber below; about rotten logs or *débris* (Europe and America).  
*F. gummosa* Fr.
41. Pileus crust-like to alutaceous-fulvous, 5-10 cm. broad; gills broad; odor strong and bitter; gregarious by roadsides in mixed forests (Europe and America).  
*F. lupina* Fr.-Ricken.
41. Pileus isabelline, 5-7 cm. broad; solitary in forests; gills subdistant, broad; on dead wood (western United States) ..... *F. permollis* Murrill.
42. Pileus hygrophanous, small, not over 5 cm. broad ..... 43.
42. Pileus not hygrophanous ..... 45.
43. Gills narrow ..... 44.
43. Gills broadly adnate, narrowed in front; pileus 2-4 cm. broad, glabrous, subferruginous (moist), buff (dry); stem tough, concolor, 3-6 mm. thick; spores  $7-8.5 \times 4-5 \mu$ ; on *débris* in woods ..... *F. rigida* Pk.
44. Growing in pastures; pileus subferruginous (moist), yellowish (dry), glabrous, 2-5 cm. broad; stem equal, hollow, reddish-brown; spores  $7.5-10(11) \times 5-6 \mu$ ; "in pastures."  
*F. halliana* Pk.
44. Growing on trunks of apple trees or rotten wood; pileus ferruginous and glabrous (moist), alutaceous and rimose-scaly (dry), 2-4 cm. broad; stem stuffed-hollow, 4-6 mm. thick, pale rust-color; spores  $10-12(14) \times 5-7 \mu$  (Europe).  
*F. azyrna* Fr.-Ricken.
45. Plants in dense caespitose clusters ..... 46.
45. Plants single, gregarious, or subcaespitose; gills crowded and narrow ..... 47.
46. Spores  $7-9 \times 4-5 \mu$ ; pileus 4-8 cm. broad, "apricot-yellow" (Ridg.), fibrillose-subscaly on margin; stem 6-10 cm. long, 3-8 mm. thick, densely fibrillose-cortinate; gills broad, at first pallid or tinged yellowish, then "ochraceous-tawny" (Ridg.); on Alnus, birch, etc., stumps and trunks (Europe and America) ..... *F. alnicola* Fr.
46. Spores  $5-6.5(7) \times 3-3.5(4)$  (Rea & Romell.); pileus 2.5-5 cm. broad, straw-color, becoming greenish, silky; stem 5-6 cm. long  $\times$  4-10 mm. thick, yellowish, white-floccose and scaly; base becoming rusty-red; gills narrow and crowded, at first whitish, then olivaceous; on trunks and wood (Europe) ..... *F. ochrochlora* Fr.
47. Pileus 5-8(10) cm. broad; solitary ..... 48.
47. Pileus 2-5 cm. broad, "antimony-yellow" to "ochraceous-buff" (Ridg.), glabrous; gills subdecurrent by tooth, soon "ochraceous-buff" to rusty; stem 2-5 cm.  $\times$  2-5 mm., flavescent; spores suboval,  $7-8.5 \times 4-5 \mu$ ; odor penetrating, taste bitterish; about stumps (eastern United States) ..... *F. flavidella* Murrill.
48. Stem 2-4 cm. long, 4-6 mm. thick, yellow; gills yellow, changing to brown where wounded, edge beaded with drops; pileus subglabrous, tawny; spores oval,  $6-7.5 \times 3.5-5.5 \mu$ ; on decaying wood (*F. expansa* Pk.) ..... *F. multifolia* Pk.

48. Stem 10-12 cm. long, 8-10 mm. thick, white, glabrous; gills pale cinnamon; pileus glabrous, pale yellowish-tan; spores 8-10.5 x 5-6.5  $\mu$ ; base of living maple tree (eastern United States) ..... *F. ludoviciana* Murrill.

### Comments on Selected Species of Flammula

Since certain species have a distinctly bitter taste or a penetrating odor, these characteristics will be especially noted below.

*Flammula alnicola* Fr. At least two different species are masquerading under this name in Europe. One possesses cystidia, in the other cystidia are lacking. Ricken's plants under this name are something else, since neither his description, which includes cystidia, nor his figures agree with the account of Fries. Specimens, however, from Bresadola and Romell, now at the New York Botanical Garden, have all the ear marks of the genuine Friesian species. The latter have no cystidia. I have collected this species in the Adirondack Mountains. The pale lemon-yellow to apricot-colored pileus, the broad gills, and long cespitose stems are among the distinguishing characters; the stem is densely fibrillose in the growing condition, and the fibrils and the lower part of the stem darken in a characteristic manner to "raw sienna" or dull "ochraceous tawny" (Ridg.), which persists as a distinctive character in the herbarium specimens. The pileus is not viscid, although moist and slightly slippery to the touch, and its margin is fibrillose. The odor is somewhat aromatic, and the taste varies from mild to bitterish.

Ricken united Bulliard's species *F. amara*, with a bitter taste and a strong penetrating odor, with the Friesian species. It appears that we may have this plant in the United States also; at least I have met with plants which fit Ricken's species. It seemed desirable, therefore, to include *F. amara* in my synopsis.

*Flammula astragalina* Fr. I have collected this both in Sweden and in the mountains of Washington and Oregon. The western specimens are quite typical and also agree with Murrill's recently described *F. laeticolor*. The pileus is "bittersweet-orange" (Ridg.) in color, or "flame scarlet" on the disk. This red-colored cap is unique. Ricken describes cystidia, but neglects to say whether they occur on the sides of the gills in his plants. The Swedish plants have no cystidia, merely sterile cells on the gill edge. The taste is bitterish, odor none.

*Flammula breandlei* Pk. There is no doubt in my mind that *F. pulchrifolia* Pk. is identical. The principal issue remaining unsolved is the question of whether by any chance these two species are *Pholiota aeruginosa* Pk. In this connection I may say there is no question that some of the collections in the Peck herbarium under *F. pulchrifolia* belong to this *Pholiota*. The type specimens of both, however, do not look quite like dried *P. aeruginosa*. Overholts (15), who has recently redescribed this *Pholiota*, does not mention pink or purplish hues on the pileus, a coloring definitely given by Peck for his two *Flammulae*. The gills of the two forms are apparently also distinctive. In the *Flammula* they are markedly broad at the region of attach-

ment. In the *Pholiota* the pileus is dry and becomes diffracted-scaly; in the *Flammula* it is said to be slightly viscid, although soon dry. In the *Flammula* the taste is said to be bitter and unpleasant. The spores are alike; cystidia are lacking in both; the gills at maturity become bright ochraceous-orange; and both occur on coniferous wood.

*Flammula carbonaria* Fr. This species seems to be widely distributed, but less common than ordinarily supposed. *F. highlandensis* is certainly closely related to it, both by its habit of sometimes growing on charred wood *débris* and by its cystidia and spores. However, *F. highlandensis* differs from *F. carbonaria* in its metalloïd cysts, although the extent to which these may occur in other species is still an open question. I have observed a slight tendency to produce such cysts in an otherwise good *F. carbonaria*. *F. highlandensis* grows in other situations more frequently and is at times solitary. I have no genuine *F. carbonaria* collected in Michigan, but have collections from Maryland and North Carolina, the latter communicated by Professor Coker. The cystidia are typical *Flammula* cystidia, lanceolate, ventricose above the slender pedicel, and measure about  $50-60 \times 9-14 \mu$ . The length given for them in the North American Flora must be considered an error. Odor none, but with a peculiar bitterish taste. (See remarks under *F. highlandensis*.)

*Flammula condensa* Pk. I have this from the Medicine Bow Mountains of Wyoming, where it grew on logs of lodgepole pine. The type specimens came from pine woods in the District of Columbia. The gills are broadly adnate-subdecurrent. It differs from the "*spumosa*" group in the more variable and extreme size of its spores. The stem is at first yellowish, becoming "old gold" (Ridg.), at length sordid brownish-rusty downwards. *It has a strong, pungent odor*, which was naturally not sensed by Peck because he received his specimens from a distance.

*Flammula comissans* Fr. This is a well marked species not mentioned by Murrill or by Peck. It was collected twice by me near Professor F. C. Stewart's camp at Seventh Lake in the Adirondack Mountains. Both lots grew at or near the base of a yellow-birch trunk, one of them alive. The spore print is characteristic, "cinnamon-rufous" (Ridg.) against the yellow caps. On white paper a thin layer is more brownish; a thick layer more reddish. The size and shape of the spores are also distinctive; they are subfusiform and measure  $9-12 \times 4-5(5.5) \mu$ . Fries says the odor is acid; Ricken, that it has no odor. The plants collected by me had a subspicy odor.

*Flammula decorata* Murrill. This is also a well marked species which I have collected in Oregon on the fallen branches of some hardwood. The size of the cystidia is not correctly given by Murrill, as shown by an examination of the type specimens with which my collections agree. They are abundant, lanceolate, tapering upward from the widest portion, with short pedicel, hyaline, thin-walled, and measure  $65-75 \times 10-15 \mu$ . This species

belongs to the fuscous-spored group. The taste is slowly bitter and somewhat nauseous. It has no special odor.

*Flammula flavida*. This species, although considered by Peck to occur in the United States, is not yet definitely placed among our species. Murrill (14) omits it, and I have no specimens which I am able to place in it. According to European accounts, it is one of the larger *Flammulae*.

*Flammula flavidella* Murrill. The plants are about the size of *Naucoria geminella* Pk., which Murrill (14, p. 200) has transferred to *Flammula*; in my judgment this species is clearly a *Naucoria*, differing from *F. flavidella* by its slender cartilaginous stems, the emarginate gills, and a more scattered habit of growth. The spore sizes of *N. geminella* and of *F. flavidella* are the same. This *Flammula* has been collected around Ann Arbor. The pileus is "antimony-yellow" to "ochraceous buff"; the stems are stuffed, then hollow, fleshy-fibrillose, pruinose at apex, subglabrous elsewhere; the odor is rather penetrating, specific; the taste is distinctly bitter. The plants were quite caespitose and were growing on rotten logs. There are no cystidia present, but the edges of the gills are provided with slender, cylindrical sterile cells. The spores of the type specimens are obscurely echinulate, broadly elliptical to suboval,  $7-8(8.5) \times 4-5 \mu$ , slightly smaller than given by Murrill.

*Flammula graveolens* Pk. This species, like *F. condensata*, differs from the "*spumosa*" group in its somewhat larger spores. It is to be considered a segregate of *F. spumosa*. It has the strong, penetrating odor of *F. condensata*, but differs from this species in its smaller, less variable spore size, generally larger pileus, and stouter stems. In *F. condensata* the gills are broad and subdistant; whether they are closer and narrower in *F. graveolens* is not clear. The cystidia of the type specimens of these two species are alike. Except for the difference in spore size, the other differential characters might very well vary to the extent given in the descriptions of the two. It seems to occur much more frequently than *F. spumosa*.

*Flammula gummosa* Fr.-Ricken. This species occurs in frondose woods, on decayed wood and *débris*. Its microscopic characters are at once different from the usual cystidia-bearing *Flammulas*. The basidia are  $22-25 \mu$  long, 4-spored, and the short cystidia scarcely project above them, varying in length from  $30-50 \mu$ , somewhat ellipsoid or ellipsoid-acuminate in shape. Besides the spore-bearing basidia and cystidia, one finds the usual basidium-like sterile elements of the hymenium—the "paraphyses" of Buller (2). A few other species with similar unaccentuated cystidia have been placed among the cystidia-bearing group in my synopsis. The viscid pileus may or may not retain the spot-like scales. The flesh is scissile, indicating its hygrophanous character. The edges of the gills are floccose-crenulate. The taste and odor is slight or none. [See further Kauffman (11) and Ricken (21)].

*Flammula halliana* Pk. There is a colored drawing of Peck's with the

type material. At the first view of this, one is reminded of *Cortinarius sphaerosporus* Pk. It is, however, not at all that species, but the impression remains that this species is a better *Cortinarius*, especially because of the hygrophanous pileus, which is said to be substriate on the margin when moist. Its decurrent gills are the main objection to placing it in that genus, although some good *Cortinarii* have a tendency towards decurrent gills. The gills and the dry pileus are pale yellowish buff. The spores are roughish, broadly elliptical, and rusty brownish-ochraceous,  $7.5-10(11) \times 5-6 \mu$  in size. There are no cystidia.

*Flammula highlandensis* Pk., *F. ascophora* Pk., and *F. pusilla* Pk. are to be considered identical from their general characters and from the unique bodies which are borne in the hymenium. These are well illustrated by the figure given by Dr. Peck (New York State Museum Report 24, Pl. 3, fig. 5), and remind one of certain metalloid bodies in the hymenium of other basidiomycetes. They are sac-like with granular or blocked-out content, projecting very slightly above the basidia except when fully developed, apparently with a rather narrow pedicel below. Peck thought that ascospores were produced within these bodies and that these were the normal spores of this plant. Later he recognized his error and considered it a form of *F. highlandensis* (N. Y. State Mus. Rep. 50: 139). He did not, however, as far as I know, find the same bodies in the type of *F. highlandensis*. Although less prominent in the latter material, probably because of the younger age of the plants I examined, they can nevertheless be made out as a definite character of this species. Further, they are also present in the hymenium of the type plants of *F. pusilla*. This uniformity of occurrence is not likely explainable on the supposition that a parasitic fungus is present in the gills of this species. I believe them to be a normal development belonging to the life history of the species. Murrill (14) refers here also *Naucoria subvelosa* Murrill and *Hebeloma Peckii* House, but I have not studied the types of these two. *F. highlandensis* occurs in Michigan. It was referred to (11, p. 488) as "*F. carbonaria* Fr. var." Cystidia of the normal type vary in number from few to fairly abundant, mostly near the edges of the gills.

*Flammula inopoda* Fr. What I feel quite confident is this species of Fries, I have from the Rocky Mountains in Colorado (Kauffman, 12). There is also a good example of it in the Atkinson Herbarium, from New Brunswick, Canada. It appears to have been unrecognized in this country, and is apparently rare. As shown in the figures of Fries (Icones, Pl. 118, fig. 1), it has the habit of *F. astragalinus*, and like the latter occurs on coniferous wood. The stems of the American plants are rather long and slender, tapering downward to the point of attachment, and in age becoming fuscous downward; the gills are "smoke-gray" to "light-grayish-olive" (Ridg.), tinged sordid olivaceous in age; the pileus is colored "warm buff" except on the deeper yellowish center, somewhat moist, not viscid; cystidia are lacking or are represented by scattered apiculate cysts; the spores

measure  $6-7.5(8) \times 4-4.5 \mu$ , rusty-ochraceous under the microscope. Ricken (21) considers Fries' species merely a variety of *F. fusa*. Rea (20) gives a description of it, but whence comes the tradition that its spores are "purple" he does not say. The spores of our plants are certainly not purplish, even though the gills possess gray shades.

*Flammula lata* Murrill. Specimens of what I take to be this species are in the Atkinson Herbarium, with complete notes on the fresh plants. There seems to be a viscosity on the stem, which Murrill overlooked. The spores are rather variable in size. The stem is solid and the plants are large, characters which would separate the species from the "*spumosa*" group. It may grow in subcespitose clusters as well as solitary. The Ithaca plants grew on dead willow trunks.

*Flammula lenta* Fr. The exact limitations of this frequent European species are not quite clear. Following Quélet, Rea (20) excludes it from *Flammula*, making it a synonym of *Hebeloma glutinosa* Fr., but this point of view is strongly objected to by Ricken (21). Murrill includes it as an American species, and considers *F. betulina* Pk. a synonym. We have an American plant which agrees entirely with the account of Fries (6); it has slender and toughish stems, and its spores measure  $5-6.5 \times 3-4 \mu$ . The gills are at first white, later greenish-ochraceous, and finally clay color with a slight incarnate-subpurplish tint; the spores, however, are very pale under the microscope, tinted smoky-ochraceous. It grows on much-decayed wood remnants and *débris* in coniferous forests. *F. betulina* Pk. has both a different habitat and habit; it occurs on logs of white birch and is usually a much stouter and more compact plant. Its spores are distinctly wider and subreniform. In both the pileus is pale grayish-buff and decorated with white veil remnants on the margin. Apparently *F. betulina* is less glutinous in wet weather than *F. lenta*. The cystidia of the two species are very similar, rather stoutly ventricose, with a stout, obtuse, subcylindric neck and a slender pedicel. The odor and taste of both are mild or nearly so.

*Flammula liquiritiae* Fr. This seems to be rare in this country, or is it perhaps frequently confused with other species? Fries has left the impression in his figures in *Icones* (Pl. 119, fig. 1) that it is a long-stemmed species; his own descriptions, however (6, 7) do not bear this out. The stem is rather slender, but rarely over 5 cm. long. I have it from the Medicine Bow Mountains of Wyoming, on coniferous wood. This collection fitted well into the description given by Ricken, except that the sterile cells on the edges of the gills ("cystidia" as called here by Ricken) are filiform, capitate, and hyaline, measuring  $35-40 \mu$  in length, both on the sides and on the edges of the gills. The gills are broadly adnate-subdecurrent and 6-8 mm. or more in width. It is known, in addition, by the absence of a cortina, by the glabrous cap and stem, and by the orange-fulvous pileus. Murrill (14) does not mention it.

*Flammula lubrica* Fr. This European species is omitted by Murrill



(14). However, we have in this country a species so close to the descriptions of Fries, and so well illustrated by his figures (Icones, Pl. 116, fig. 1) that it is doubtless the same. Microscopically it differs from Ricken's conception of that species by the slightly longer spores. The only other species it might be referred to is *F. subfulva* Pk., but I have been unable to distinguish clearly between these two, further than Peck himself did. I have collected the species mentioned above as *F. lubrica*, in the Adirondack Mountains, as well as in the mountains of Colorado and Washington. The pileus is "ochraceous-orange" to "zinc-orange" (Ridg.) and tawny on the disk. The cortina is usually well developed, leaving remnants on the margin of the pileus and a cortinate, evanescent zone at the apex of the fibrillose stem. The cystidia are rather characteristic,  $50-65(70) \times 10-15 \mu$  in size, thick-walled in mature plants, and many of them with a somewhat irregular or wavy, stout neck. The spores measure  $5-6(7.5) \times 3.5-4 \mu$ . The plants are usually larger than most *Flammulae*.

My notes show that there is a radish-like odor present in greater or less degree; sometimes the odor is slight, at other times strong and penetrating. All these collections grew on or about coniferous logs or *débris*. Fries does not emphasize its habitat in connection with conifers. Nor is it possible with all these data to draw a line, with reference either to the odor or to the wood relations, between *F. lubrica* and *F. subfulva*. The latter, apparently, is a smaller plant, but the type collection is not sufficient to establish this point. This species, by its pale gills and spores, verges towards the genus *Hebeloma*, and when not attached to wood, as sometimes happens, might well be looked for in that genus.

*Flammula magna* Pk. This and *Cortinarius validipes* Pk. are undoubtedly identical. Only mature or overmature plants were studied by Peck, and the species needs further attention when fresh young stages are found. The type plants of both look like large rusty-yellow *Cortinarii*, but the gills are truly decurrent and narrow. The veil characters if any are entirely unknown, but a veil of some sort is indicated by the fibrillose stems. The spores are exactly the same in both collections, broadly ellipsoid, minutely tuberculate, rusty-yellow under the microscope, and measure (8)  $9-10 \times 5-6 \mu$ . Cystidia are lacking.

*Flammula mixta* Fr. The plants collected by me at North Elba, Adirondack Mountains (10), and referred to this species, are something else. The species is so far unknown in this country.

*Flammula malicola* nov. nom. This is *F. sulphurea* Pk. (*non* Massee). Murrill has made *F. sulphurea* a synonym of *F. alnicola*. There are many similarities in the dried herbarium specimens of the two, but I believe that Peck rightly kept it separate. His reasons are given in his comments (N. Y. State Mus. Bull. 157: 26. 1912), viz.: "from *F. alnicola*" it differs "by its place of growth, white flesh, and viscid, hygrophanous pileus." He might have added that it is also more markedly umbonate and brighter in color;

the gills are narrower, and the spores slightly larger than in *F. alnicola*. When dried the viscid surface of the pileus has become markedly rugose-wrinkled, and the umbo prominent. Its "odor and taste is disagreeable" (Peck). *F. alnicola marginalis* Pk. appears to be a form of the same species.

*Flammula multifolia* Pk. The spores were said by Peck to be subglobose,  $4-5\ \mu$  in diameter. They are, however, oval to elliptic-oval, measuring  $6-7.5 \times 3.5-5.5\ \mu$ , and are rounded at one end; some of them resemble corn kernels in shape. *F. expansa* Pk. is the same.

*Flammula ochrochlora* Fr. This species seems hitherto to have been unknown in the United States. Specimens from Sweden in the Atkinson Herbarium agree closely with the account given by Rea (20), especially in their microscopic characters. The gills of the dried Swedish specimens are "tawny olive" to "snuff brown" (Ridg.). Material which I collected in the Adirondack Mountains is very probably this species, although the dried gills are somewhat darker.

*Flammula penetrans* Fr. Murrill has placed here what I consider, in part at least, to be the Friesian species *F. sapinea*. *F. penetrans* has a glabrous, not a floccose-scaly pileus, and the colors are different. Material from Romell, in the Atkinson Herbarium, is without doubt the Friesian species. The spore characters given by Ricken (21) and Rea (20) are obviously not from their own studies but copied from Saccardo, and the size quoted is  $8-9 \times 4-5\ \mu$ . This is also the spore size of *F. sapinea*. In the Swedish specimens mentioned above, the spores are characteristic in size and shape; they measure  $6-7.5 \times 3.5-4.5\ \mu$  and tend to be ventricose in shape. The spore sizes as given by Saccardo for the basidiomycetes are well known to be undependable.

*Flammula permollis* Murrill. The type of this species has spores which are almost hyaline under the microscope, but lying against the gills they show a pale ochraceous color. It is insufficiently described.

*Flammula polychroa* Berk. For a full account of this see Kauffman (11, p. 484).

*Flammula sapinea* Fr. In the older American lists this was nearly always reported, largely because the conifer substratum was supposed to be all-sufficient. Murrill (14) excludes it altogether and in its place gives *F. penetrans* the honor of a common American distribution. In my own field experience I have found no facts to support Murrill's opinion. I still consider *F. sapinea* Fr. a good American species. Fries' plate (Icones, Pl. 118, fig. 3), to be sure, shows luxuriant plants, and he speaks of it as a "noble species"; but, although one ordinarily finds only small-sized plants, rarely a few large specimens do appear. Very few species of conifer-loving *Flammulae* have a scaly pileus, and this one is easily recognizable. Only one or a few scattered plants are ordinarily seen in a place, although they may be caespitose at times. It has a rather strong odor. If I am correct in this diagnosis, then *F. eccentrica* Pk. becomes a synonym. An examination of

the decayed wood remnants found with the type of *F. eccentrica* showed that it grew on coniferous wood. I have frequently seen *F. sapinea* with both deformed and eccentric stems, especially when growing caespitose. This feature is referred to in a broad way by Fries (6) when he says the stems are "difformis."

*Flammula spumosa* Fr. This and the closely related species can be called "the *spumosa* complex." The group consists of *F. spumosa*, *F. graveolens*, *F. squalida*, *F. subflavida*, and *F. piceina*. When the growing history and constancy of certain characters are fully known, it may be possible to differentiate clearly between these species. At present the dividing lines are vague and many necessary details are not recorded. *F. graveolens* is fairly well differentiated from *F. spumosa* by its odor and slightly different spores, as well as by a number of minor characters; it is probably a segregate of the old European *F. spumosa*. *F. squalida* is not well known. The two species of Murrill need further study before they can be distinguished. Rea (20) gives the spore size too large.

The colors of flesh, cap, and stem are much used in this group, but are very confusing. The parts may be sulfur-yellow, greenish-yellow, dull yellow, etc., but the shades vary with the age of the fresh plants when picked; there is always a tendency towards a fulvous or tawny color on the disk of the pileus, and the stems, especially the lower portion, may be fuscous, ferruginous, or brunnescent. Data on a sufficient number of collections, showing just how the gills are attached, their width and spacing, are not at hand. All the collections appear to have a stuffed to hollow tubule in the stems. They all have a manifest cortina; in *F. spumosa* this is regularly pale greenish-yellow according to Fries (6). A complete account of the stages at time of collecting may show that colors and color changes are constant for each form, but for several of the group I have not been able to make such notes.

*Flammula squalida* Pk. By its subcartilaginous stems, this species verges towards the genus *Naucoria*; otherwise, however, it belongs to the "*spumosa*" complex. (See under *F. spumosa*.)

*Flammula subflavida* Murrill. This also belongs to the "*spumosa*" complex; as in the species previously mentioned, the cortina is at first fibrillose-interwoven so as to appear almost membranous. (See *F. spumosa*.)

*Flammula subfulva* Pk. Peck points out that this may be only a form of *F. lubrica* (N. Y. State Mus. Rep. 50: 136). It is said to differ from the latter species in the uniform color of the pileus and the grayish-white flesh. The type has cystidia with slender necks, which are wavy in outline as in *F. lubrica*. It should perhaps be excluded. (See remarks under *F. lubrica*.)

*Flammula subviridis* Murrill. This is a very distinct species which I have collected in the Cascade Mountains east of Seattle. The type specimens were sent by Miss McKenny from Olympia, Washington, and were accompanied by three colored drawings, from which the species is readily

recognizable. It is very different in its habit and characters from *Pholiota aeruginosa*, having a long tapering or rooting stem, without a cortina even in the young stage. The whole young plant is at first covered by a whitish pulverulence which may be considered as a sort of universal veil, and which at length disappears, especially on the stem. *F. picrea* Fr. is clothed in the same manner, and these two species are related. Individuals vary in size; sometimes the mature pileus measures only 4-5 cm. while the stem is 5-7 cm. long; at other times the plants reach the size given by Murrill. The pileus, stem, and gills are "pyrite-yellow" to "warbler green" (Ridg.), but may become deeper-colored in age, the gills at length being stained by the ferruginous spores. The taste is intensely bitter; the odor, none. *F. ochrochlorus* Fr. differs by its cortina and somewhat scaly stem, and by its smaller size and paler colors. The spores of the two seem to be alike.

*Flammula sulphurea* Pk. (See *F. malicola*.)

*Flammula unicolor* Murrill. The spores of the type specimens measure 7-9 x 4-5  $\mu$  (not 10 x 6  $\mu$  as given by Murrill). The cystidia are abundant.

*Flammula velata* Pk. In this species, according to Peck (Bull. Torrey Bot. Club 30: 96), "the strongly developed veil is a prominent character of the species." The type specimens came from Idaho. The cystidia are abundant, ventricose-sublanceolate, 50-60 x 10-13(15)  $\mu$ , hyaline, with a slender pedicel; the surface is longitudinally striate, the outline undulate. The spores are broadly elliptical, 6.5-7.5(8) x 4-5  $\mu$ , and are yellowish under the microscope. "Taste mild."

*Flammula vialis* Murrill. This western species seems to differ sufficiently from the "*spumosa*" group by its solid stem, non-cespitose habit, and scattered cystidia. Although the pileus was described as dry, the type specimens indicate that it had at least a subviscid, separable pellicle. The cystidia are mostly found at and near the edges of the gills, are subventricose with a cylindrical neck and slender pedicel, hyaline, and measure about 60-70 x 10-14  $\mu$ . The spores are ellipsoid in one view, plano-convex in the other, and measure 6.5-7.5 x 3.5-4  $\mu$ . The specimens grew on a railroad tie which may have been of coniferous wood.

#### Synonyms and Excluded or Doubtful Species of *Flammula*

Species known only from the British Isles: *F. aldridgei* Masee (syn. *F. veluticeps* Cke. & Masee). *F. carnosa* Masee. *F. clitopila* Cke. & Smith. *F. decipiens* W. G. Smith. *F. filicea* Cke. *F. floccifera* B. & Br. *F. inaurata* W. G. Smith. *F. juncina* W. G. Smith. *F. nitens* Cke. & Masee. *F. purpurata* Cke. & Masee.

*Gymnopilus Abramsii* Murrill = *Cortinarius* sp.

*F. aliena* Pk. = *Paxillus* sp., probably *P. scambus* (Fr.) Quél.

*F. anomala* Pk. = *Paxillus anomala* (Pk.) comb. nov.

*F. apicrea* Fr. Not known at present.

*Gymnopilus aromaticus* Murrill = *Pholiota* sp., probably a form of *P. muricata* Fr.

*F. ascophora* Pk. = *Flammula highlandensis* Pk.

*F. austera* Fr. Not known at present.

*Gymnopilus autumnalis* (Pk.) Murrill (= *Agaricus autumnalis* Pk. N. Y. State Mus. Rep. 23: 92. 1873). Peck changed the name to *Pholiota autumnalis* in 1908. Murrill (14), in his account of the *Flammulas*, gives it the name mentioned above. Overholts (15), finally, makes it a synonym of *Pholiota marginata*. In the N. Y. State Mus. Bull. 157: 9. 1912, Peck reports a fatal case of poisoning from eating what was supposedly this mushroom. Mary Whetstone, M.D., of Minneapolis, Minn., who sent the data quoted by Peck, has several times kindly sent me collections of this noxious form from the original locus. The spores of this harmful species are too narrow and the gills are too broad for it to be considered *P. marginata*. The taste is bitter, becoming disagreeable in the mouth; it is quite strong and nauseous. The cortina is not very marked, although the stem is cortinate-fibrillose. The plants grew on rotten sticks and *débris* in a lumber yard. This western species is therefore probably undescribed, but until my notes on the fresh plants are fuller it is better to leave it without a name. A fuller account of its poisonous qualities has appeared (Kauffman, 11, p. 854).

*Gymnopilus bellulus* (Pk.) Murrill = *Naucoria bellula* (Pk.) Sacc.

*F. bresadolae* Schulz. Unknown in America.

*F. californica* Earle. Probably a *Hebeloma*.

*F. decurrens* Pk. The type was not seen and is probably not in existence. It is omitted by Murrill (14).

*F. decussata* Fr. Not known at present.

*F. echinulispora* Murrill = *Cortinarius* sp.

*F. eccentrica* Pk. = *Flammula sapinea* Fr.

*F. edulis* Pk. = *Hebeloma edule* (Pk.) comb. nov.

*F. expansa* Pk. = *Flammula multifolia* Pk.

*Gymnopilus fagicola* Murrill = *Pholiota fagicola* (Murrill) comb. nov.

*F. farinacea* Murrill = *Cortinarius farinaceus* (Murrill) comb. nov.

*F. filia* Fr. Not much known about this species.

*Gymnopilus foedatus* (Pk.) Murrill. This was described by Peck as a *Hebeloma*. It should be *Cortinarius foedatus* (Pk.) comb. nov.

*Gymnopilus geminellus* (Pk.) Murrill = *Naucoria geminella* (Pk.) Sacc.

*F. granulosa* Pk. = *Pholiota muricata* Fr.

*F. helomorpha* Fr. = *Paxillus helomorphus* (Fr.) Quél.

*F. hillii* Murrill = *Naucoria bellula* (Pk.) Sacc.

*F. laeticolor* Murrill = *Flammula astragalina* Fr.

*F. lenta* Fr. = *Hebeloma glutinosum* Fr.

*Gymnopilus longisporus* Murrill = *Paxillus longisporus* (Murrill) comb. nov.

*Gymnopilus oregonensis* Murrill = *Naucoria oregonensis* (Murrill) comb. nov.

- F. pallida* Murrill. Probably a *Hebeloma*.  
*F. praecox* Pk. = **Cortinarius praecox** (Pk.) comb. nov.  
*F. pulchrifolia* Pk. = *Flammula braendlei* Pk.  
*F. pusilla* Pk. = *Flammula highlandensis* Pk.  
*F. rubicunda* Rea. Why not a *Cortinarius*?  
*F. scamba* Fr. = *Paxillus scambus* (Fr.) Quél.  
*F. sphagnophila* Pk. = **Tubaria sphagnophila** (Pk.) comb. nov.  
*F. spinulifer* Murrill = *Psilocybe* sp. with purple-brown spores.  
*Gymnopilus squamulosus* Murrill = **Pholiota squamulosus** (Murrill) comb. nov.  
*F. subcarbonaria* Murrill = *Cortinarius* sp.  
*F. tammii* Fr. = *Phylloporus rhodoxanthus* (Schur.) Bres.  
*F. Tricholoma* (A. & S.) Quél. = *Paxillus Tricholoma* (A. & S.) Fr.  
*F. viridans* Fr. = *Pholiota aeruginosa* Pk.  
*F. viscida* Pk. = **Pholiota viscida** (Pk.) comb. nov.  
*F. viscidissima* Murrill = *Cortinarius* sp.

#### PAXILLUS

The natural relationships of the genus *Paxillus*, as it has come down to us, are such that the species which have been placed in it, or which probably should be placed in it, link the genus on different sides to *Clitocybe*, *Clitopilus*, *Flammula*, *Inocybe*, and the *Boletaceae* respectively. What was true when Fries (7, p. 401) remarked that "the genus is not yet properly defined" remains true today.

The generic characters that stand out in the traditional usage are: (a) the anastomosing gills, a character which shows relationship to the *Boletaceae*, especially *Phylloporus rhodoxanthus* (Schw.) Bres. (see Kauffman, 10); (b) the discrete hymenophore and therefore the ease with which it is separable from the trama of the pileus, a character not infrequent in the genus *Clitocybe*; (c) the decurrent gills common to *Clitocybe*, *Clitopilus*, and *Flammula*; and (d) the color of the spores, which varies over a considerable range, being either almost white or with ochraceous or flesh tints or, in some specimens, yellowish to rusty-colored, thus showing connections with *Clitopilus* and *Flammula*.

Fries (7) separated the eccentric and central-stemmed species and placed them in his tribes *Tapinia* and *Lepista* respectively. Without going further into the interim history of the group, it will serve my purpose sufficiently to discuss only a few of the recent attempts to handle the species of this group. Rea (20) places all the older species of *Paxillus* as well as *Phylloporus rhodoxanthus* in this genus, and includes it in the *Boletaceae*. This arrangement seems to me to do violence to the essential characters of the families concerned. To my mind, Ricken (21) has approached the matter in a much more effective way. Here we find what is equivalent to a division of the genus *Paxillus* into four subgenera; *Paxillus* Fr., *Clitopilus*

Fr., *Tapinia* Fr., and *Ripartites* Karsten. However, whatever one does with the old genus *Clitopilus*, it is quite unlikely that all the even-spored species of that genus which are now known could be made to fit naturally into this grouping under *Paxillus*. On the other hand, I believe that for the present Ricken's procedure with regard to the other three subgenera is a step in the right direction. It will perhaps give us an understanding of a number of troublesome species—species which have always been outcasts, and whose place in any classification was never assured.

If we attempt now to arrange the better-known species by an approximate use of Ricken's three subgenera, we shall be able to place certain species hitherto ambiguous elsewhere into proper alignment with each other.

The following outline, therefore, is presented as a temporary arrangement of the species;

- I. *Paxillus* proper. Spores elliptical to subfusiform. Gills sinuate-subdecurrent.
  - P. sordarius* Fr.
  - P. extenuatus* Fr.
  - P. longisporus* (Murrill) Kauff.
- II. *Tapinia*. Spores elliptical. Gills decurrent, forked or anastomosing.
  - P. corrugatus* Atk.
  - P. pannuoides* Fr.
  - P. atrotomentosus* Fr.
  - P. involutus* Fr.
- III. *Ripartites*. Spores globose to subglobose, minutely rough or smooth. Gills adnate to decurrent.
  - P. Tricholoma* (A. & S.) Fr.
  - P. helomorphus* Fr.
  - P. scambus* Fr.
  - P. alienus* (Pk.) Kauff.
  - P. anomalus* (Pk.) Kauff.

### Comments on the Species of *Paxillus*

*Paxillus alienus* (Pk.) comb. nov. This is a rare species. It was described originally from Mt. Gretna, Pennsylvania, from specimens growing on partly buried anthracite coal. I collected it in the same locality in 1924, growing on humus or wood remains, and, although I have no specimens from elsewhere, I feel sure I have also seen it rarely in other parts of the country. It usually grows solitary, and not necessarily on coal. The pileus is "grayish-olive-buff" (Ridg.), almost subviscid or merely moist when fresh, with decurved margin, 2–4 cm. broad. The gills are decurrent, narrow, and crowded. In Peck's description the gills are said to be "subdistant," but I think this is doubtful. The stem is concolorous and glabrous except at base. The spores, like those of the type specimens at Albany, are globose, minutely echinulate, 3–4  $\mu$  in diameter, the size given by Peck being too large. There are no cystidia. This species may be *P. scambus* Fr., but further collections and notes are necessary. *P. scambus* is described as having adnate gills.

**Paxillus anomalus** (Pk.) comb. nov. The type specimens of this have globose, smooth spores, 4-6  $\mu$  in diameter. The spores are subhyaline under the microscope, with a slight smoky-ochraceous tint, certainly not "brownish-ferruginous" as given in Peck's description. It is poorly known, but is separable from *P. alienus* by its smooth spores, its umbonate-infundibuliform pileus, and its "cespitose" habit.

*Paxillus atroamentosus* Fr. is well known (see manuals).

*Paxillus corrugatus* Atk. is fully described by its author.

*Paxillus extenuatus* Fr. This is not with certainty known as occurring in the United States, but I suspect that I have had it on several occasions.

*Paxillus helomorphus* Fr. Not known in America.

*Paxillus involutus* Fr. In the coniferous regions of America. Not abundant.

**Paxillus longisporus** (Murrill) comb. nov. This was described by Murrill (14, p. 207) as a *Flammula*. It is well marked by its long, subfusiform spores and by the distinct olive-greenish hue in the dried type specimens. The spores measure longer than the length given by Murrill. They are oblong-fusiform, subhyaline, slightly tinged with ochraceous, 9-12  $\times$  3.5-4.5  $\mu$  in size, and the epispore is slightly and longitudinally wrinkled under the high power of the microscope. Cystidia are present although hard to find; they are not at all of the *Flammula* type, 70-85  $\times$  14-16  $\mu$ , hyaline and smooth. By its spores the species seems to be related to *Clitopilus orcella*, but in other respects it is very different and its position is very probably within this group. Its characters generally bring it close to *P. sordarius* Fr.

**Paxillus panuoides** Fr. Although infrequently found, I have one collection from nearly every part of the United States visited. It often grows in dark places, caverns, on coal-mine timbers, and in similar situations, although not exclusively so.

**Paxillus scambus** Fr. Not definitely known in America. (See remarks on *P. alienus* (Pk.).)

**Paxillus sordarius** Fr.-Ricken. I have what I interpret as this species from the Adirondack Mountains and from Colorado. The spores are narrow, subelliptic, pointed at one end, almost subfusiform, hyaline, 8-9(10)  $\times$  3.5  $\mu$ . There are no cystidia present. The gills are easily separable from the hymenophore; they are soon "avellaneous" to "wood-brown" (Ridg.), at first obtusely adnate, becoming sinuate-decurrent, crowded and moderately broad, almost narrow at times. The pileus is 5-8 cm. broad, varying in color from "pinkish buff" to "cinnamon buff," becoming sordid, and, like the flesh, mottled with watery streaks. The stem is rather stout, 5-6 cm. long, 7-10(12) mm. thick, often clavate-enlarged below or abruptly short-pointed at the very base, spongy-stuffed, concolor, becoming sordid-streaked. Odor and taste slight, sometimes subaromatic and subnauseous. Solitary or subcespitose, on the ground,



especially on banks along mountain roads, probably mostly from decayed wood remnants in the soil.

*Paxillus strigosus* Pk. and *Paxillus strigiceps* Fr. appear to be *P. Tricholoma*.

*Paxillus Tricholoma* (A. & S.) Fr. This species has been placed both in *Flammula* and in *Inocybe*. It was not originally described under the old genus *Agaricus*. Its subglobose, minutely echinulate spores are a pale ochraceous color and measure  $4 \times 3 \mu$ . There are no cystidia. The pileus is noted for its strigose-ciliate margin, which is 2-4 cm. broad; stems are 2-4 cm. long  $\times$  3-6 mm. thick, usually smaller rather than the full size given here.

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## FALSE POLYEMBRYONY IN MAIZE<sup>1</sup>

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In some cases the corn embryo has multiple plumules and primary roots, with only a single cotyledon. Since there are not two independent embryos but only a single abnormal embryo, this condition has been called false polyembryony. Though such cases are evidently very rare, they have been reported by a number of observers.

The first case reported was by Schrenk (1894), who illustrated and briefly described two kernels each of which had two plumules and two primary roots and a single cotyledon. Two similar seeds were found by Goss and described by Kempton (1913). Weatherwax (1921) found and described several cases of false polyembryony in maize. In each of two cases the grain produced two stalks and two primary roots. In both these kernels the coleoptyle and coleorhiza were duplicated, but there was only one cotyledon. In another instance three stalks were found united to a single cotyledon.

In connection with germination tests of corn at the Nebraska Experiment Station, some kernels showing false polyembryony have been found which are illustrated in Plate III. Figure 1 shows a normal germinating kernel of corn with the single plumule still enclosed in the coleoptyle and the single primary root emerging from the coleorhiza. The cotyledon is imbedded in the kernel. Figure 2 shows a case of false polyembryony. There is a single cotyledon, but there are two plumules each with its own coleoptyle, and two primary roots enclosed in a single coleorhiza. This kernel was planted and grown to maturity, producing two normal ear-bearing stalks of identical appearance. These ears were selfed and two generations were grown to determine whether the peculiarity might be transmitted, but no further abnormalities appeared. Figure 3 is a similar kernel found last year and grown to maturity, likewise producing two identical stalks and ears. These ears were selfed but the seeds have not yet been germinated. The embryo of another kernel of this type which had been germinated was sectioned to show the relation of the double parts to the cotyledon. This cotyledon had two main fibrovascular bundles where a normal cotyledon has but one, as shown in figures 8 and 9. Figures 4 and 5 are two successive growth stages from another kernel exhibiting an abnormal embryo. This embryo had a single cotyledon and a single

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coleoptyle with a single plumule, but had two primary roots within a single coleorhiza. The mesocotyl had a double stele, as suggested by the slight median crease shown in figure 5 and fully disclosed in figure 6, which latter is a photomicrograph of a cross section taken about midway of its length. A series of cross sections demonstrated that this double vascular system gradually separated below into the two distinct root systems, while it merged into a normal scattered vascular system at the basal node of the stalk. Nothing double was observed about the stalk or leaves except a slight notch at the tip of the first leaf which is shown in figure 5. Figure 7 is the cross section of a normal mesocotyl for comparison.

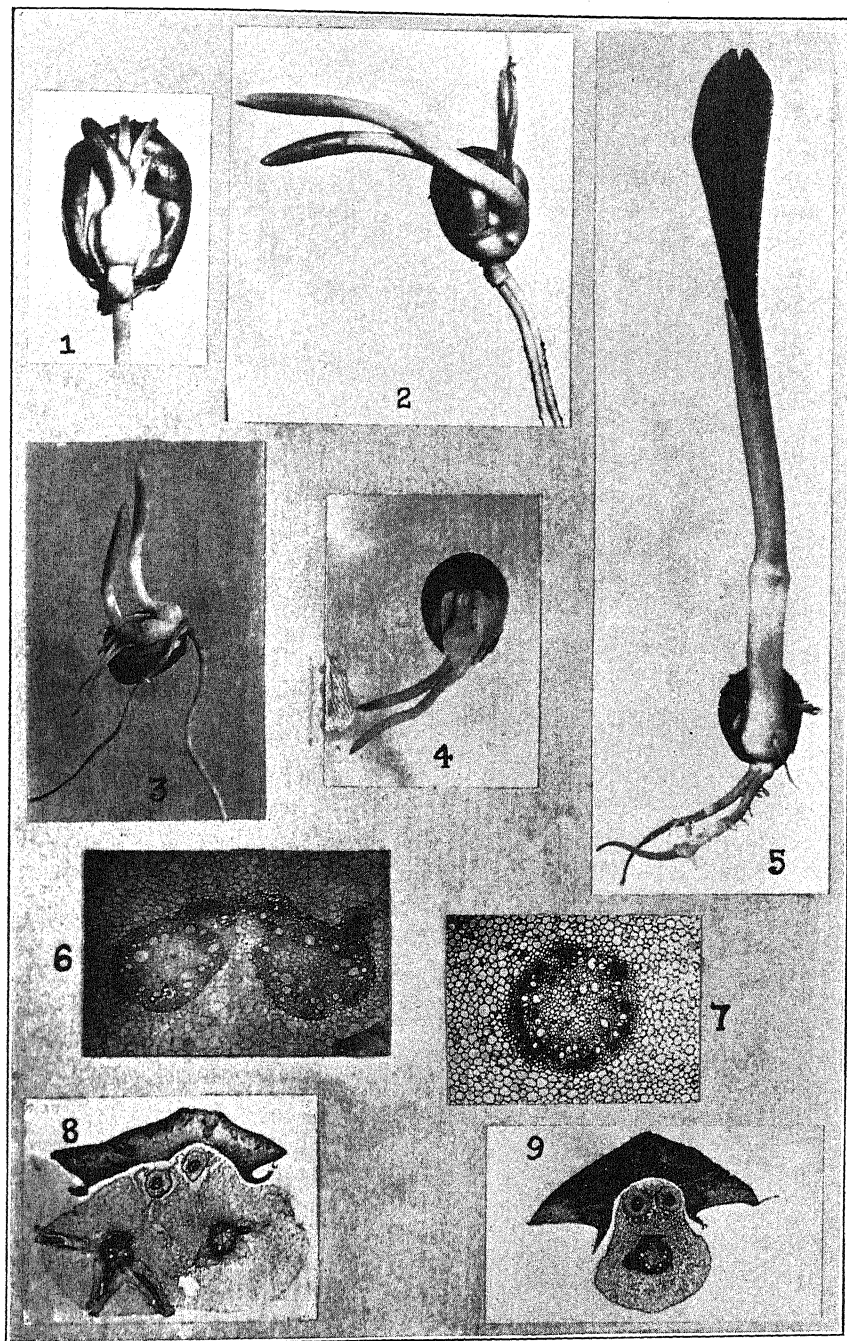
The fact that such embryos have but a single cotyledon each, as well as the fact that where they have been grown they have produced identical stalks, indicates that the two plumules both arose from a single fertilized egg. At some stage of development the embryo must have given rise to two growing points instead of one wherever the parts are doubled.

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#### EXPLANATION OF PLATE III

- FIG. 1. Normal kernel germinating.  
FIGS. 2, 3. Germinating kernel with two plumules and two primary roots.  
FIG. 4. Germinating kernel with two primary roots and a single plumule.  
FIG. 5. Same kernel as in figure 4, later stage.  
FIG. 6. Photomicrograph of cross section of mesocotyl showing double vascular system of seedling of figure 5.  
FIG. 7. Cross section of a normal mesocotyl for comparison with figure 6.  
FIG. 8. Photomicrograph of cross section of germinated embryo at the point of attachment of the cotyledon, showing two stalks and a single cotyledon. (Two main fibro-vascular bundles are shown in the cotyledon, whereas the normal cotyledon has but one.) This embryo was of the same type as that shown in figures 2 and 3.  
FIG. 9. Cross section of germinated normal embryo for comparison.



KIESELBACH: FALSE POLYEMBRYONY



## FASCIATED KERNELS, REVERSED KERNELS, AND RELATED ABNORMALITIES IN MAIZE<sup>1</sup>

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Each pistillate spikelet of corn normally has two flowers, the upper of which gives rise to the kernel while the lower remains sterile and soon degenerates. Sometimes both flowers function, each producing a kernel, the one from the lower flower being reversed with its germ turned toward the butt of the ear instead of toward the tip. This is very common in Country Gentleman sweet corn as noted by Stewart (1915), and the resultant crowding leads to the irregular arrangement of the kernels in that variety. Sometimes the upper flower remains sterile while the lower gives rise to a reversed kernel. Two of these occur near the tip of the ear shown in figure 1, Plate IV. When both flowers of the spikelet function the two kernels formed may be united, forming the so-called "fused" or "fasciated" kernels as seen near the tip of the ear shown in figure 2. Such fusion may vary from slight union to so complete fusion that the two kernels appear to be a single kernel with two embryos.

### HISTORICAL

A kernel with two embryos was described and illustrated by de Petit-Thouars (1810).<sup>2</sup> This may have been either a case of very complete fusion, or, as he thought, two seeds developed in a single fruit. He considered this as a reversion to a primitive condition in which the fruit had more than one seed and thought it would account for the double style in corn.

Kempton (1913) described a number of cases of inverted kernels and fused kernels. He accounts for the reversed kernels by the development of the lower, normally sterile, flower. When both flowers of a spikelet develop, two kernels are formed back to back which sometimes fuse, making connate kernels.

Two kernels with two embryos each were found by Bohutinsky (1914) in 1909, and another in 1912. He thought that some of such cases were single kernels in which two ovules had developed, while others were due to the fusion of two kernels.

<sup>1</sup> Contribution from the Department of Agronomy, University of Nebraska. Published with the approval of the director as paper no. 9, Journal Series, Nebraska Agricultural Experiment Station.

<sup>2</sup> This case was also described in his "Histoire d'un morceau du bois," page 85, 1815, and was referred to in P. J. Turpin's "Esquisse d'organographie végétale," page 20, 1837.

Wolfe (1916) found two fused kernels which appeared like normal kernels except for the presence of an extra germ, and for a slight line of demarcation which extended around each kernel parallel to the embryos. More recently such kernels have been reported by Blaringhem (1920, 1924), who described a variety of corn, *Zea mays* var. *polysperma*, in which such kernels are common. Miss Stratton (1923), working with the same variety, has studied these kernels further. In this variety the germs are sometimes directed toward the sides instead of toward the ends of the ears.

TABLE I. *Corn Varieties, Strains, and Hybrids in which Fasciated Kernels were Found. Crop Grown at the Nebraska Experiment Station in 1924*

Variety	Seed Source	No. Fused Kernels Found
<i>Dent Varieties</i>		
Hogue pure line no. 8	Nebraska	40
Nebr. White Prize pure line no. 733	Nebraska	45
Minnesota 23	Minnesota	30
Rustler's White Dent	Minnesota	28
Cornell 11	New York	16
Thayer Yellow Dent	Washington	14
Cattle Corn	Nebraska	10
Minnesota 13	Minnesota	8
Reid Yellow Dent	Nebraska	8
Nebraska White Prize	Nebraska	5
U. S. Selection 133	U. S. Dept. Agr.	5
Yellow Dent	Antelope Co., Nebr.	9
Yellow Dent	Dawes Co., Nebr.	4
Hogue Yellow Dent	Nebraska	4
Brookings 86	South Dakota	2
Yellow Dent	Lincoln Co., Nebr.	2
Yellow Dent	Hall Co., Nebr.	2
Yellow Dent	Cherry Co., Nebr.	1
<i>Varieties other than Dent</i>		
Country Gentleman	Nebraska	21
White Australian Flint	Nebraska	3
Yellow Flint	Connecticut	2
<i>F<sub>1</sub> Variety-hybrids</i>		
Blair White × Iowa Gold Mine	Nebraska	14
Thayer Yellow Dent × Rustler's White Dent	Nebraska	12
Hogue Yellow Dent × Blue Mexican Sweet	Nebraska	6
Ramosa × Sweet Corn	Nebraska	11
Cornell 11 × Nevada White Dent	Nebraska	3
Substation White × Hogue Yellow Dent	Nebraska	2
Substation White × Bloody Butcher	Nebraska	2
Rustler's White Dent × Hogue Yellow Dent	Nebraska	2
Leaming × Substation White	Nebraska	2
Hogue Yellow Dent × Nebr. White Prize	Nebraska	4
U. S. Selection 133 × Nebr. White Prize	Nebraska	4
U. S. Selection 133 × Substation White	Nebraska	1
Neal Paymaster × Hogue Yellow Dent	Nebraska	1
White Australian Flint × University no. 3	Nebraska	1
Country Gentleman Sweet × White Pearl Pop	Nebraska	1

A systematic search at the Nebraska Experiment Station for fasciated or fused kernels has disclosed that they are of rather frequent occurrence. This abnormality has been found in the 38 varieties, inbred pure lines, and crosses listed in table 1. In an average run of corn approximately one kernel in 200,000 was fasciated, whereas in some varieties this abnormality proved 20 times as frequent. Fused kernels, misplaced germs, and reversed kernels all occur most frequently near the tips of the ears, as shown in figures 1-3.

Four fused kernels and four kernels having their germ on the edge are shown in the middle and lower rows respectively of figure 4 in comparison with normal kernels in the upper row of the same figure. The reverse side of these same kernels is shown in the same order in figure 5. A normal kernel with its one embryo and a fasciated kernel with an embryo on each side are shown germinating in figure 6.

The four fused kernels shown in figure 4 were sectioned and studied in detail microscopically in order to establish the nature and histological origin of the double feature. Diagrammatic drawings of these are given together with a normal kernel in figures 7-11. The arrangement of pericarp, aleurone layer, endosperm, and embryo is shown. The degree of fusion in fasciated kernels differs greatly in different cases, as brought out in these drawings. In some the separation is almost complete with a mere medial point of union. In other cases the two kernels are so completely fused that only a line marks the place of union. In some cases this line is due to a failure of the endosperms of the two fused kernels to fit snugly against each other rather than to incomplete fusion of the pericarps. Varying with the degree of fusion, fasciated kernels differ in the extent to which the pericarps, seed coats, and aleurone layers develop in the region where fasciation has been effected. In the case of only slight union, these parts are usually prominent. Where very close union has been effected, their development in the intervening area may have been entirely arrested and a mere line of demarcation be visible between the two merging endosperms. The two halves of a fasciated kernel may differ in endosperm type and color according to the inheritance carried by the gametes involved in fertilization.

A low power photomicrograph of a partially fused kernel taken approximately one week after fertilization is shown in cross section in figure 12, Plate V. The relatively very thick pericarp, which in this case is fully developed between the two endosperms, is characteristic of this stage of growth. The medial area of this section where fusion has occurred is shown under higher magnification in figure 13.

Another type of double kernel in which the pericarp does not extend between the endosperms is illustrated by the microscopic views in figures 15 and 16. (The folds in the pericarp of figure 15 are mechanical flaws accompanying the sectioning of the young kernel.) There was no definite external or internal proof of the fusion of two kernels. The separation of



the two endosperms by their aleurone layers and the seed coats which have fused is evidence that two ovules were involved. A rather similar condition is shown in the vertical section of a young kernel illustrated in figure 14. It may be possible that both ovules had developed within a single pistil rather than within two fused pistils. So many gradations of unmistakable fusion have been found, however, varying from slight to complete, that even such cases as these may result from fusion of two originally independent pistils. Fusion commonly takes place in the very early floral development of the grain before the separating palets have made much growth.

No evidence was found to explain why some kernels have their embryos laterally on one edge as shown at the tip of the ear in figure 2, Plate IV. According to Miss Walker (1906), the grass pistil consists of three carpels, two of which give rise to the style and the third bears the ovule. In the corn flower the carpel directed towards the base of the ear usually produces the ovule, but if for any reason the ovule should arise from one of the other carpels it would perhaps be turned so as to give the embryo a lateral position.

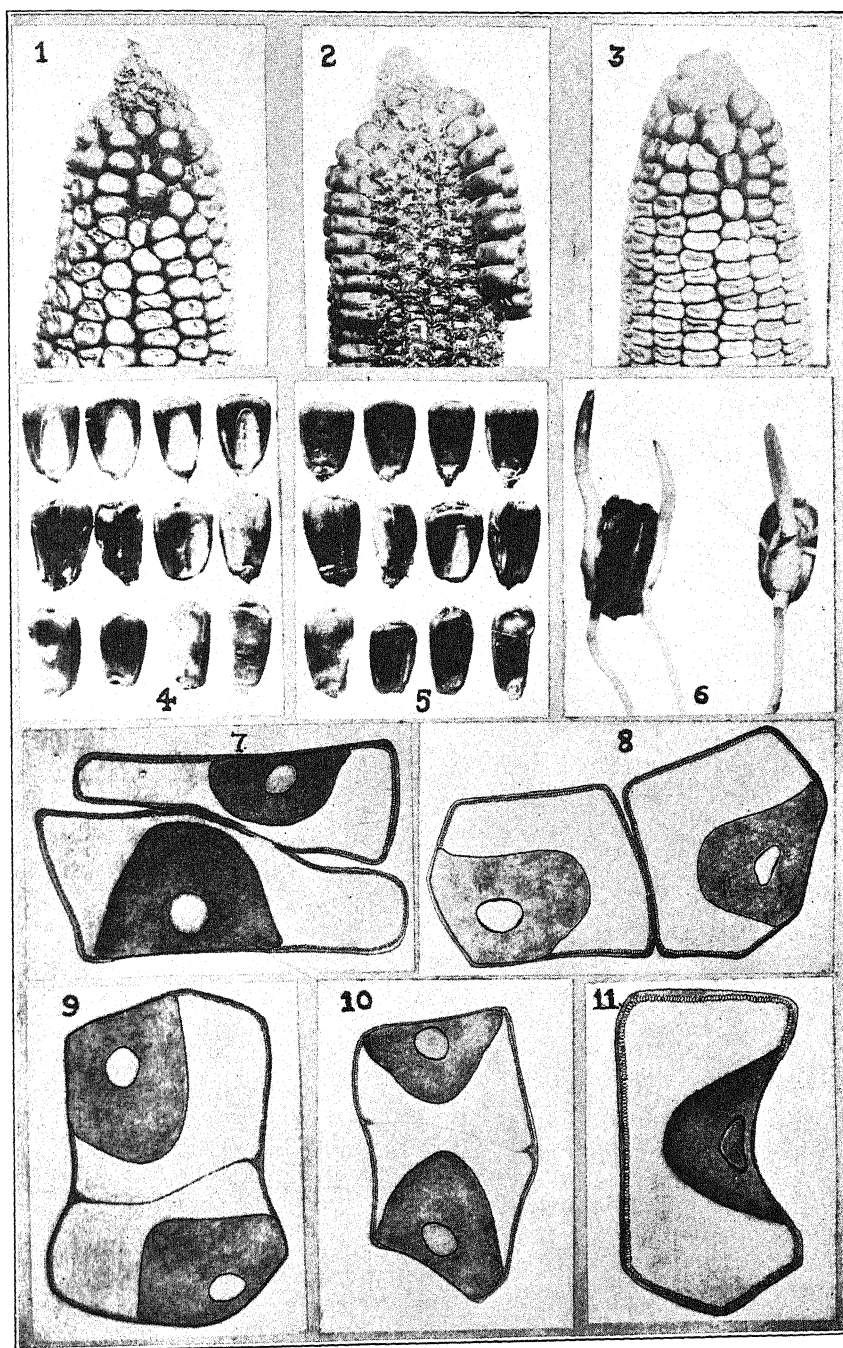
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#### EXPLANATION OF FIGURES

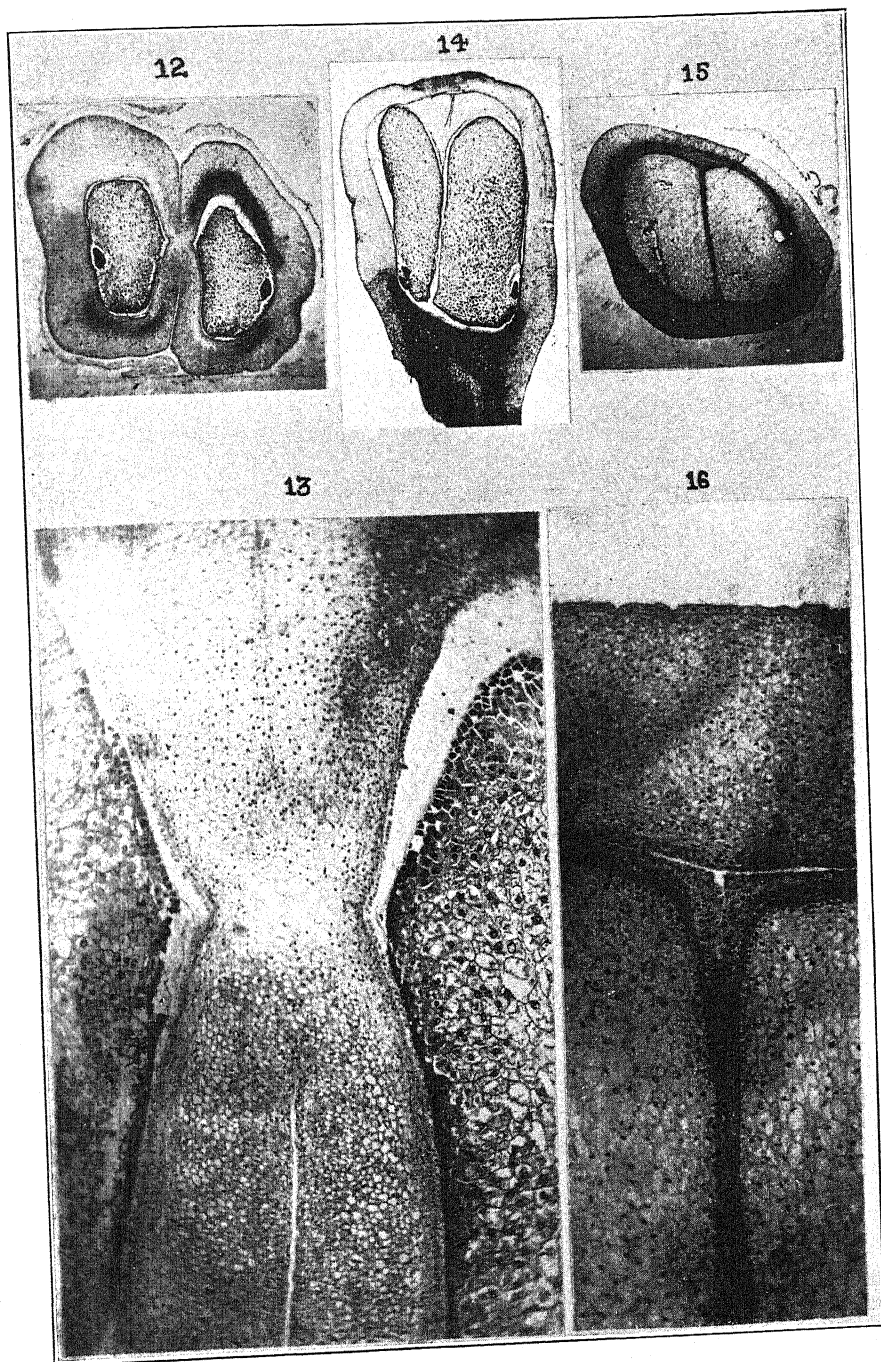
##### PLATE IV

- FIG. 1. Part of ear showing 2 reversed kernels near tip.
- FIG. 2. Part of ear showing 2 tip kernels at the right with embryo on edge.
- FIG. 3. Part of ear showing fused kernel near tip.
- FIG. 4. Top row, normal kernels; middle row, fused kernels; lower row, kernels with embryo on edge.
- FIG. 5. Same kernels in the same order as in figure 4, but with opposite side exposed.
- FIG. 6. Fused kernel germinating, and normal kernel for comparison.
- FIGS. 7-10. Drawings of cross sections of the mature fused kernels shown in figures 4 and 5, middle rows.
- FIG. 11. Drawing of cross section of normal mature kernel.

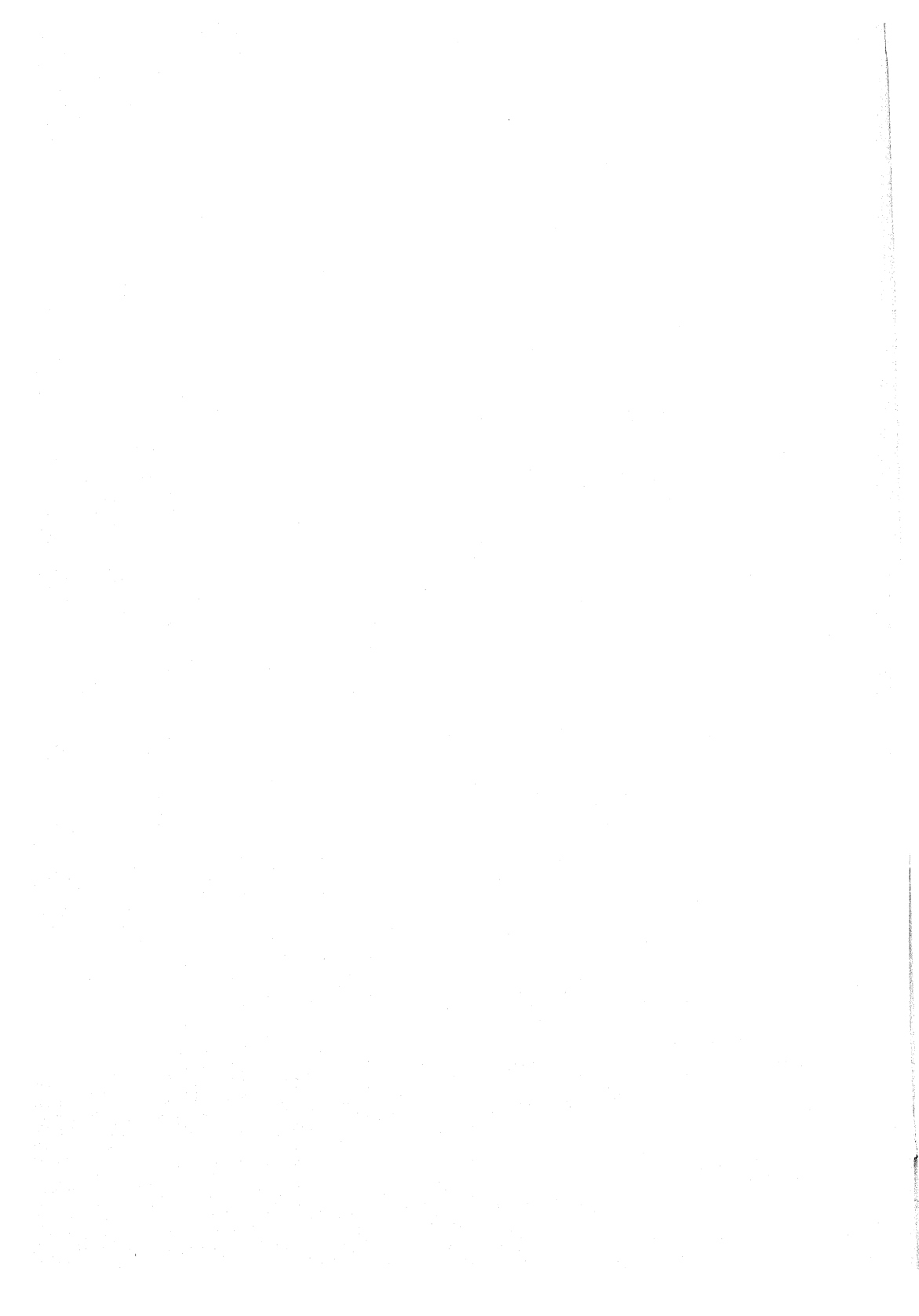


KIESELBACH: ABNORMALITIES IN MAIZE





KIESSELBACH: ABNORMALITIES IN MAIZE



## PLATE V

- FIG. 12. Photomicrograph of cross section of young fused kernel (slightly fused).  
FIG. 13. Median part of same section shown in figure 12 under higher power, showing fused region.  
FIG. 14. Photomicrograph of longitudinal section of young fused kernel.  
FIG. 15. Photomicrograph of cross section of young kernel (complete fusion).  
FIG. 16. Part of same section under higher power, showing continuous character of the fused pericarp.

# EFFECTS OF CERTAIN ACIDS AND THEIR SODIUM SALTS UPON THE GROWTH OF *SCLEROTINIA CINEREA*

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## INTRODUCTION

Various studies have been made of the toxic effects of certain acids upon diverse plant and animal material, and in some cases the investigators have tried to account for the toxic action observed. The results are somewhat conflicting. Wolf and Harris (18), working with *Bacillus perfringens* and *B. sporogenes*, came to the conclusion that hydrochloric, lactic, formic, butyric, acetic, malic, and succinic acids affect the growth of *B. perfringens* in proportion to the hydrogen-ion concentration. The anions and undissociated acids behave merely as foreign bodies possessing a certain inhibiting influence in proportion to their molecular concentration. From their studies of the action of formic acid and certain salts upon *B. typhosus*, Norton and Hsu (13) concluded that the disinfecting power of an acid is approximately proportional to the hydrogen-ion concentration. Krönig and Paul (10), as a result of their investigations on the effect of a series of compounds, organic and inorganic, upon *Bacillus anthracis*, state that there is a general relationship between the disinfecting power and the amount of dissociated hydrogen present with the acids employed. However, certain investigators find that the toxic action of certain acids may be attributed in large part to other causes than the hydrogen ion. Clark (2), who used the hanging-drop method to study the toxic effect of certain deleterious agents upon the germination and subsequent development of *Aspergillus flavus*, *Sterigmatocystis nigra*, *Oedocephalum albidum*, *Penicillium glaucum*, and *Botrytis vulgaris*, found that the toxic properties of acetic acid are to be attributed almost wholly to the undissociated molecule. Collett (4) worked with *Paramoecium caudatum*, *Stylonichia pustulata*, *Euplotes patella*, and *Vorticella nebulifera*. She observed that the hydrogen ion is not the only factor in toxicity, for, in solutions of equal pH, the acids used were not equally toxic. Depression of ionization showed that the anions of formic, acetic, propionic, butyric, valeric, citric, benzoic, phthalic, and salicylic acids are toxic to *Paramoecium* and *Euplotes*. She also found that mixtures of certain acids (formic, acetic, butyric, and valeric) with non-toxic concentrations of their salts are more toxic than can be accounted for by the pH of the mixtures. The same thing applied to salicylic acid when mixed with a dilute solution of the sodium salt. This seems to indicate that the molecules are in themselves toxic. Kahlenberg and True

(9) investigated the effect of certain acids upon the growth of the roots of seedlings of *Lupinus albus* L. Their results obtained with acetic and butyric acids show that the undissociated acid is toxic. In another paper, True (15) reported that in inorganic acids the purest H-ion effect is seen, while in those of the fatty acid series, which dissociate much less freely, the undissociated molecule plays an important rôle often exceeding that played by the H ions. In general, the anions of organic acids possess relatively slight toxic properties. Winslow and Lochridge (17) investigated the disinfecting power of hydrochloric, sulfuric, acetic, and benzoic acids upon the typhoid and colon bacilli. Their investigations led them to conclude that the toxicity of the two inorganic acids is due principally to the H ion. In the cases of acetic and benzoic acids, the toxic factor is apparently the anion or the undissociated molecule—the latter, being so much greater in amount, probably playing the principal part.

It is extremely difficult to draw definite conclusions concerning the relative effects of various acids on plants from much of the literature because of the varied material, the methods of study, and the absence of exact hydrogen-ion determinations, especially in the older writings. In general, however, there is indication that the toxic effects of the mineral acids hydrochloric and sulfuric are due chiefly to the hydrogen ion. In the case of the usual organic acids employed, the toxic action seems to be due to the combined influence of the H ion, the anion to a slight degree, and the undissociated molecule.

Another problem that is opened by the investigations of the toxicity of an acid is that of the limiting hydrogen-ion concentration for the growth of an organism. There are numerous references to these limits in the literature, and only a few will be cited here. Taylor (14), using four different kinds of bacteria, found indications that among the common organic acids there is a great variation or specificity in their activity toward the organisms used. Wolf and Harris (18) gave as the mean critical pH values for the seven acids used with *B. perfringens*, pH 4.82, and with *B. sporogenes*, pH 4.94. In their work on the roots of the seedlings of *Lupinus albus* L., Kahlenberg and True (9) found 1/6400 M per liter the limit for apparently normal growth with hydrochloric, phosphoric, sulfuric, formic, and salicylic acids. Acetic acid had a limit of 1/1600 M per liter and butyric acid one of 1/3200 M per liter. Much of the work has been conducted, however, using a nutrient solution to which varying amounts of some one acid were added. Johnson (8) found the following critical pH values, using hydrochloric acid: *Mucor glomerula*, pH 3.2–3.4; *Aspergillus terricola*, pH 1.6–1.8; *A. oryzae*, pH 1.6–1.8; *Penicillium variabile*, pH 1.6–1.8; *P. italicum*, pH 1.9–2.2; *Fusarium bullatum*, pH 2.0–2.2; and *F. oxysporium*, pH 1.8–2.2. Fred and Loomis (6) used a mannitol solution as a culture medium and adjusted its reaction by the addition of sterile N/10 sulfuric acid. There was no growth of *B. radiculicola* when the pH of the culture medium was 2.7 or over. In



their study of the reaction of soil solutions, Morgan and Gruzit (12) found that an acid reaction of the soil solution adjusted by means of a mineral acid had a distinct toxic effect at N/1200 on the growth of bacteria.

It is the purpose of this investigation to determine whether the hydrogen ion is always the chief factor of toxicity in the effect of various acids upon the germination and growth of fungous spores, or, if not, to indicate a possible toxic factor or factors involved. It is thought that, at the same time, work of this nature will throw much light upon the limiting hydrogen-ion concentrations for the organism used when grown in the presence of certain acids, and will also show the changes in the condition of the nutrient solution by the reaction of the organism. For this purpose the following experiments have been planned, using six acids which have frequently been employed by various other investigators.

#### METHODS

*Organism.* The organism employed in the following investigations was the brown-rot fungus, *Sclerotinia cinerea* (Bon.) Schröt. This fungus was chosen because of its ready fruiting and rapid growth, and was obtained from the Bureau of Plant Industry through the kindness of John A. Roberts. By means of the microloop (5), a colony was obtained from a single-spore inoculation of peach decoction. The spores used in the investigations recorded in this paper were all descendants of the same single spore.

*Culture Solution.* The culture solution used was a modified Richards' solution. *Sclerotinia cinerea* grew upon it excellently and produced abundant spores. The composition of the culture medium employed was as follows:

Extract of 100 g. of dried peaches per liter;  
Cane sugar, 20 g. per liter;  
Peptone, 2 g. per liter;  
 $\text{Ca}(\text{NO}_3)_2$  0.5 M, 4 cc. per liter;  
 $\text{MgSO}_4$  0.5 M, 2 cc. per liter;  
 $\text{KH}_2\text{PO}_4$  0.5 M, 4 cc. per liter;  
Distilled water, 1000 cc.

Six hundred grams of dried peaches were cut up and boiled gently in two liters of water for two hours. The peach juice was decanted, and then the pulp remaining was reboiled for one hour with about one liter of water. The decanted juice was then added to the first decanted juice. Next the peaches were pressed in a fruit press, and then the assembled juice was filtered while hot through cotton and made up to three liters. The other ingredients were then added, and the solution was autoclaved for fifteen minutes at fourteen pounds. In order to lower the acidity of the solution, N/1 NaOH was added until the culture medium reached in most cases a pH of 5.2. Consequently, because of the varying amounts of sodium hydroxid it was necessary to add, the mean total volume of the stock culture solution was 3285 cc.  $\pm$  0.8 percent. That the presence of the sodium hydroxid had no appreciable deleterious effect is shown by observing the growth curves (text fig. 1).

Stock cultures of the fungus were grown on 3 percent peach-agar slants. Inoculations (except in one instance later mentioned) were always made with spores from eleven-day-old cultures which had grown on 3 percent peach-agar slants, care always being taken that the size of the tubes was the same and that the angle of slant was also the same. In this way, differences in age of spores and in the amount of available growing surface were avoided.

150-cc. Erlenmeyer flasks were used for fungus-growth. Into each one of these was placed 25 cc. of the nutrient solution of twice the concentration, and distilled water and the acid or salt to be studied were added in varying proportions as required to make a total volume in each flask of 50 cc. Each series was always run in duplicate and often in triplicate. The series of flasks containing the medium under consideration was made at the same time and always as near to the day of inoculation as possible. The flasks were tightly plugged with cotton and autoclaved for fifteen minutes at fourteen pounds.

A duplicate culture was made for each step in a particular series. These duplicate flasks, instead of being inoculated, were used for obtaining the initial hydrogen-ion concentration of the particular step in the series after autoclaving.

At the time of inoculation, 10 cc. of sterile distilled water was added to the inoculation tube containing the fungus growing on 3 percent peach agar. The culture tube was then carefully rotated between the hands for about three minutes. The water containing the detached spores was re-decanted into the sterile tube which had originally contained it, and was again rotated for three minutes to secure separation of the spore groups. A platinum loop, whose size was not changed during the various series, was used for inoculation. One loopful of the inoculum was added to each of the Erlenmeyer flasks. After the entire series had been inoculated, the flasks were placed in the same dark closet and the fungus was allowed to grow for ten days. The contents of the flasks was then filtered, and the filter papers and flasks were washed at least three times with distilled water. In certain cases, for each step in a series, the nutrient solution upon which the fungus had grown was filtered directly into clean flasks. In such cases, no additional water was allowed to run into the flasks by the washing of the filter paper. Four drops of toluol were added, the flasks were tightly plugged with cotton, and their pH was determined on the following day. After the flasks containing the solutions for hydrogen-ion concentration determinations had been removed from the filter rack, the filters were washed in the usual way. The filters were dried in an electric oven at 85°-95° C. for at least 24 hours, placed in a desiccator, allowed to cool, and weighed. As each filter paper had, previous to filtration, been heated in the electric oven at the same temperature for twelve hours and then placed in a desiccator and weighed when cool, it was necessary only to subtract the

dry weight of the filter from the weight of the filter plus the fungus to obtain the weight of the mycelium. Incubation was at laboratory temperature, which had a mean of about 22° C. although in one case the mean temperature reached 24.6° C. Never did the temperature go below 18° C., and only once was it recorded as high as 28° C. To obviate these differences in temperature, the results have been reported on a percentage basis with the control for each series taken as representing 100 per cent.

*Care of Glassware.* All glassware was cleaned by subjecting it twice to boiling potassium bichromate-sulfuric acid cleaning solution. It was then heated twice with boiling distilled water and rinsed two or three times in cold distilled water. Pipettes and other measuring vessels were always cleaned and standardized.

*Chemicals.* The acids and salts used in these experiments were the purest obtainable. Sulfuric acid, phosphoric acid, glacial acetic acid, and sodium dihydrogen phosphate.  $2\text{H}_2\text{O}$  were Powers-Weightman-Rosengarten analytical chemicals. Formic acid 85 percent, butyric acid C.P., sodium formate C.P., and sodium salicylate C.P. were obtained from Coleman and Bell Company. Salicylic acid and sodium sulfate.  $10\text{H}_2\text{O}$  came from Merck, sodium acetate C.P. from the J. T. Baker Company, and sodium butyrate (tech.) from the Eastman Kodak Company.

The pH values recorded in this paper were all obtained electrometrically by the use of a potentiometer. The colorimetric method was tried at first, using as standards McIlvaine's  $\text{Na}_2\text{HPO}_4$ -citric acid buffer solution (3) and adding the correct amounts of the appropriate indicators, brom phenol blue, brom cresol purple, thymol blue, etc. A comparison of the pH values derived from this method with those found electrometrically showed that the colorimetric method could not be used, as the error varied with different solutions. Undoubtedly, strong coloration and turbidity rendered any exact reading impossible.

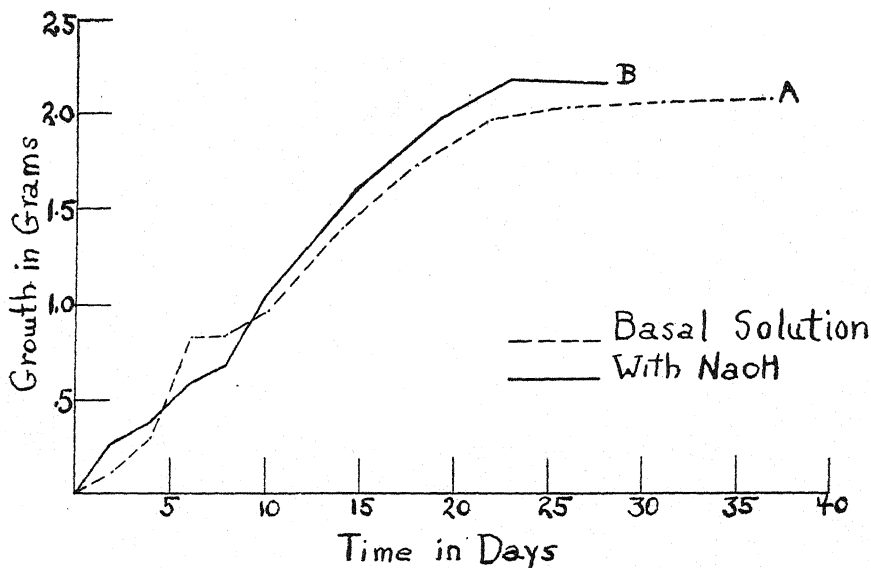
#### EXPERIMENTAL DATA

Before studying the effects of the various acids and salts upon the growth of *Sclerotinia cinerea*, it was necessary to determine the growth of the fungus in the culture medium to which no N/1 sodium hydroxid had been added, and then to compare this result with the growth of the fungus in the medium which had had its hydrogen-ion concentration lowered by the addition of the necessary amount of N/1 sodium hydroxid to bring the pH to approximately 5.35. Changes in the hydrogen-ion concentration during this growth period were also obtained. Thirty flasks containing 25 cc. nutrient solution of double concentration plus 25 cc. distilled water were inoculated with spores from a ten-day-old peach-agar slant. The flasks were harvested, three flasks at a time, as shown by text figure 1. The dry weights were obtained in the usual manner, and the average weight was calculated. From the average weights, taken at various intervals, the curve A in text figure 1 was obtained. It was found that the growth of the

fungus during the first two days was very slow, but that the rate increased and became rapid at 10 days. Furthermore, the growth curve became flattened at about the 23d day.

The changes in hydrogen-ion concentrations of this series were followed colorimetrically from day to day, but the results were later discarded upon finding that the approximate values could not be accurately determined.

Another series was therefore undertaken in which the changes in hydrogen-ion concentration were recorded. These readings were accurately obtained electrometrically. It was found that, starting with pH 3.8, there was a slight increase of acidity followed by a decided decrease. On the 16th day, the pH was 4.25.



TEXT FIG. 1. The effect of the addition of NaOH to the basal nutrient solution upon the rate of growth of *Sclerotinia cinerea*.

Text figure 1, B shows the growth curve of the series in which the nutrient solution had its hydrogen-ion concentration lowered to pH 5.35 by the addition of N/1 sodium hydroxid. It may be seen to follow approximately curve A. The addition of sodium hydroxid had therefore no appreciable effect upon the growth of the fungus. In both cases, the temperature was approximately the same, 22°–25° C. The hydrogen-ion concentration of this third series showed a rise to pH 4.4 on the 6th day, followed by a fall to pH 5.65 on the 28th day.

Other cultures in other series during the course of these experiments have shown that the hydrogen-ion concentration is changed finally to pH 7 +. One culture gave pH 7.1 at the end of thirty-two days.

From these results the following conclusions were drawn: *Sclerotinia*

*cinerea* at first raises the hydrogen-ion concentration of the medium and later depresses it above the initial, finally changing it to pH 7.0 +. The addition of N/1 sodium hydroxid to lower the hydrogen-ion concentration has no appreciable deleterious effect under the conditions of the experiment. The fungus may be harvested at the end of 10 days' growth with the assurance that the growth rate has not decreased (text fig. 1).

The results of the various acid and salt series are shown in tables 1-17. Column 1 of each table gives the number of cc. of acid or salt used; column 2 the initial pH of the culture medium with the added acid or salt before inoculation; column 3, the percentage growth based on the control for the particular series; column 4, the final pH after the fungus had been growing on the culture medium for 10 days; column 5, the normality of the nutrient solution before autoclaving but after the addition of the proper amount of salt or acid. It is to be noted that in every case the average temperature is indicated at the head of the table.

A precipitate appeared in the sulfuric acid, phosphoric acid, and sodium phosphate cultures. Check cultures, not inoculated, were filtered, and the dry weight of the precipitate was obtained in the usual manner. This weight was then subtracted from the corresponding values of the weight of the fungus upon the filter paper.

Throughout the investigation there have been three principal causes for variation, and these must be considered in critically studying the tables. The hydrogen-ion concentration of the original peach solution varied slightly (pH 3.8, pH 3.9, pH 4.0, etc.) in the various series—thus necessitating the addition of varying quantities of N/1 sodium hydroxid. There were small differences in the growth temperature. A certain amount of each culture was lost through autoclaving and standing, although great care was taken in the use of the autoclave and the flasks were plugged as tightly as possible with cotton.

It may be seen from the tables that a slight increase in acidity is usually beneficial for growth of *Sclerotinia cinerea*. This is in harmony with the work of Webb (16), who found that *Botrytis cinerea* has the crest of its germination curve at pH 3.1. With sulfuric acid, the greatest amount of growth was obtained at pH 2.85 (table 1), with phosphoric acid at pH 3.90 (table 3), and with formic acid at pH 4.37 (table 5). One series with acetic acid gave 109 percent growth at pH 4.66 (table 7), while another series failed to give an increase above the control (table 8). In the case of butyric acid (tables 11, 12, 14) and salicylic acid (table 16), an increase in active acidity did not give an increase above the control for the series.

In almost every case, the original pH of the culture medium was changed by fungous growth. The final pH values differed for the various acids. When *Sclerotinia cinerea* was grown in the sulfuric acid solutions, the culture medium with an original acidity lower than pH 4.35 became more acid at the end of ten days. When it grew in solutions with initially high acidity,

*Experimental Data Showing the Influence of the Addition of Certain Acids and Their Sodium Salts upon the Growth of Sclerotinia cinerea*

TABLE I					TABLE 2					TABLE 3				
H <sub>2</sub> SO <sub>4</sub> (2N)					Na <sub>2</sub> SO <sub>4</sub> (2N)					H <sub>3</sub> PO <sub>4</sub> (3N/2)				
Av. T. 23° C.					Av. T. 23° C.					Av. T. 24° C.				
cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N
0	5.25	100.0*	4.50	—	0	5.25	100.0*	4.70	—	0	6.85	100.0*	4.55	—
.3	4.35	94.2	4.35	.0120	.3	5.20	108.7	—	.0120	1	5.90	102.9	4.30	.0300
.6	3.60	68.0	3.75	.0241	.6	5.30	106.3	—	.0241	2	3.90	116.6	3.90	.0602
.8	3.20	103.0	3.55	.0321	.8	5.18	97.3	—	.0321	3	3.35	106.6	3.45	.0901
1.0	2.85	118.3	3.15	.0400	1.0	—	103.9	4.70	.0400	5	2.64	78.5	2.62	.1502
1.2	2.53	70.9	2.60	.0481	1.2	—	93.5	—	.0481	6	2.55	35.3	—	.1802
1.4	2.25	33.0	2.20	.0562	1.4	5.25	95.3	—	.0562	8	2.20	0	2.16	.2409
1.6	2.00	9.6	—	.0641	1.6	—	99.7	—	.0641	11	1.95	—	1.95	.3906
1.8	1.95	8.4	1.97	.0719	1.8	—	94.2	—	.0719	13	—	—	—	.3906
2.0	1.85	0	1.85	.0800	2.0	5.25	91.3	4.70	.0800	15	1.78	—	—	.4950

TABLE 4					TABLE 5					TABLE 6				
NaH <sub>2</sub> PO <sub>4</sub> (3N/2)					HCOOH(N/1)					HCOONa(N/1)				
Av. T. 24° C.					Av. T. 22° C.					Av. T. 22.1° C.				
cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N
0	6.85	100.0*	4.55	—	0	5.20	100.0*	4.50	—	0	4.80	100.0*	4.32	—
1	6.35	90.5	—	.0300	.2	4.88	99.0	4.45	.0040	—	—	—	—	.0040
2	—	78.1	—	.0602	.4	4.60	100.3	4.53	.0080	.4	—	—	—	.0080
3	5.32	83.9	—	.0901	.6	4.37	108.2	4.51	.0120	.6	4.83	94.3	4.73	.0120
5	5.18	74.7	4.65	.1502	.8	4.20	97.1	4.45	.0160	—	—	100.0	—	.0160
6	5.11	82.2	—	.1802	1.0	4.10	86.8	4.49	.0200	1.0	4.86	99.4	4.90	.0200
8	—	—	—	.2409	1.2	3.99	44.3	4.49	.0239	1.2	4.89	100.0	4.91	.0239
11	5.04	73.3	—	.3300	1.4	3.87	0	—	.0280	—	—	—	—	—
13	—	—	—	.3906	1.6	3.82	—	—	—	—	—	—	—	—
15	4.95	62.0	4.50	.4950	—	—	—	—	—	—	—	—	—	—

Experimental Data Showing the Influence of the Addition of Certain Acids and Their Sodium Salts upon the Growth of *Sclerotinia cinerea*

TABLE 7

CH <sub>3</sub> COOH(N/t)		Av. T. 22° C.	
cc.	Initial pH	% Growth	Final pH
0	5.20	100.0*	4.50
.2	4.96	102.7	4.47
.4	4.66	109.0	—
.6	4.65	90.1	4.53
.8	4.56	76.9	—
1.0	4.50	21.6	4.55
1.2	4.45	0	—
1.4	—	—	—

TABLE 8

CH <sub>3</sub> COOH(N/t)		Av. T. 24.6° C.	
cc.	Initial pH	% Growth	Final pH
0	5.29	100.0*	4.62
—	—	—	—
.4	4.84	94.9	4.59
.8	4.65	44.4	4.57
1.0	4.54	5.6	4.55
1.2	—	0	—
—	—	—	—

TABLE 9

CH <sub>3</sub> COONa(N/t)		Av. T. 22.1° C.	
cc.	Initial pH	% Growth	Final pH
0	4.80	100.0*	4.32
.4	5.00	101.5	4.65
.6	—	100.0	—
.8	—	87.9	—
1.0	5.20	100.0	5.04
1.2	—	—	—

TABLE 10

CH <sub>3</sub> COOH(N/t)		Av. T. 23.5° C.	
cc.	Initial pH	% Growth	Final pH
0	5.65	100.0*	—
—	—	—	—
—	—	—	—
.8	4.89	78.1	—
1.0	4.70	51.1	—
1.2	4.69	0	—

TABLE 11

C <sub>3</sub> H <sub>7</sub> COOH(N/10)		Av. T. 22.1° C.	
cc.	Initial pH	% Growth	Final pH
0	4.80	100.0*	4.26
.5	4.76	61.1	4.30
1.0	4.70	61.1	4.37
1.5	4.65	53.1	4.40
2.0	4.61	10.6	4.55
2.5	4.5+	3.2	—
3.0	—	0	—

TABLE 12

C <sub>3</sub> H <sub>7</sub> COOH(N/t)		Av. T. 23.5° C.	
cc.	Initial pH	% Growth	Final pH
0	5.20	100.0*	—
.2	4.91	47.3	4.70
.4	4.79	0	—

Experimental Data Showing the Influence of the Addition of Certain Acids and Their Sodium Salts upon the Growth of *Sclerotinia cinerea*

TABLE 13					TABLE 14					TABLE 15				
C <sub>3</sub> H <sub>7</sub> COONa(N/10)					C <sub>3</sub> H <sub>7</sub> COOH(N/10)					C <sub>3</sub> H <sub>7</sub> COONa(N/10)				
Av. T. 24.6° C.					Av. T. 23.5° C.					Av. T. 23.5° C.				
cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N
0	4.70	100.0*	4.30	—	0	4.80	100.0*	—	—	0	4.80	100.0*	—	—
.5	4.74	46.7	4.15	.0010	.5	—	56.5	—	.0010	.5	—	48.6	—	.0010
1.0	—	—	—	.0020	1.0	—	52.0	—	.0020	1.0	—	41.8	—	.0020
1.5	4.79	14.2	4.45	.0030	1.5	—	0	—	.0030	1.5	—	40.7	—	.0030
2.0	4.81	4.4	4.68	.0040	2.0	—	—	—	.0040	2.0	—	4.5	—	.0040
2.5	4.83	3.6	4.77	.0050	—	—	—	—	—	2.5	—	3.4	—	.0050

TABLE 16					TABLE 17				
C <sub>6</sub> H <sub>4</sub> OHCOOH(N/100)					C <sub>6</sub> H <sub>4</sub> OHCOONa(N/100)				
Av. T. 24.6° C.					Av. T. 24.6° C.				
cc.	Initial pH	% Growth	Final pH	N	cc.	Initial pH	% Growth	Final pH	N
0	5.10	100.0*	4.34	—	0	5.10	100.0*	4.34	—
2	—	88.6	—	.0004	6	—	66.0	4.40	.0012
6	5.02	78.2	4.37	.0012	15	5.09	74.5	4.50	.0030
10	4.90	75.5	—	.0020	21	—	78.2	4.48	.0042
15	4.85	75.9	4.36	.0030	25	5.08	72.9	4.40	.0050
18	4.75	81.2	4.35	.0036					
21	4.69	56.4	3.75	.0042					
23	—	7.4	3.90	.0046					
25	4.64	4.8	4.35	.0050					

\* Mean weight of control, 0.805 g.



it lowered these as a rule. With phosphoric acid, the final acidity of those cultures whose original acidity was lower than pH 3.90 was raised, while those cultures whose original acidity was greater than pH 3.90 were either raised or lowered. With formic acid, the final pH of the culture medium varied from 4.45 to 4.51. The final pH values of the acetic-acid series varied with the series from pH 4.47 to pH 4.62. In the butyric-acid series, the final pH values of the culture medium were arranged in regular order as seen in table 11. With salicylic acid, the final pH values of the different culture media varied but were in the neighborhood of pH 4.35 as a rule.

It was extremely interesting to observe the changes that took place in the character of the mycelium as the culture medium became progressively more unfavorable for fungous growth. In most cases, as the medium became more acid there was a marked increase in the number of spores, and the mycelium, which was whitish and evenly covered the surface of the culture medium of the controls, became coated with brownish spores and irregularly distributed. As acidity increased, the amount of mycelium decreased and it formed small, more or less isolated colonies which were often raised and generally, although not always, tended to form a ring on the side of the flask. Finally, in those cultures which just permitted growth, only a few scattered colonies about the size of pin-heads were present. In certain cases (salicylic acid), the mycelium showed a tendency to wrinkle as the acidity of the medium was increased. With the fatty acids, the culture solutions became progressively redder toward the acid end of the series as a result of fungous growth. Those cultures in which *Sclerotinia cinerea* was unable to grow kept their original color.

The hydrogen-ion concentration of the first cultures which did not show growth depended upon the acids used. Thus the following values were secured: sulfuric acid, pH 1.85; phosphoric acid, pH 2.20 (although one small colony was observed at this concentration); formic acid, pH 3.87; acetic acid, pH 4.45; butyric acid, pH 4.5; salicylic acid, slightly above pH 4.64. These values with the fatty acids, as will be shown later, were dependent upon the initial acidity of the culture solution.

Tables 2, 4, 6, 9, 13, 15, and 17 give the data for the corresponding sodium salts of the acids. It was found that all the salts used gave good growth except sodium butyrate, but only in the case of sodium sulfate and sodium acetate did the growth ever exceed that of the control.

The original hydrogen-ion concentration was changed by fungous growth. In the case of sodium sulfate (table 2), it reached pH 4.70; with sodium dihydrogen phosphate, pH 4.50-4.65 (table 4). With sodium formate (table 6), sodium acetate (table 9), and sodium butyrate (table 13), the final pH values arranged themselves in a progressive series, and with sodium salicylate (table 17), the final pH values varied with the concentration.

Unfavorable conditions of the medium for fungous growth were shown by the increased amount of spore-production in the sulfate, phosphate,

butyrate, and salicylate cultures. In the case of the two latter salts, the mycelium showed a marked tendency to wrinkle as the salt concentration increased, and in the sodium butyrate cultures of higher salt concentrations it appeared much like the mycelium in the higher butyric-acid concentrations.

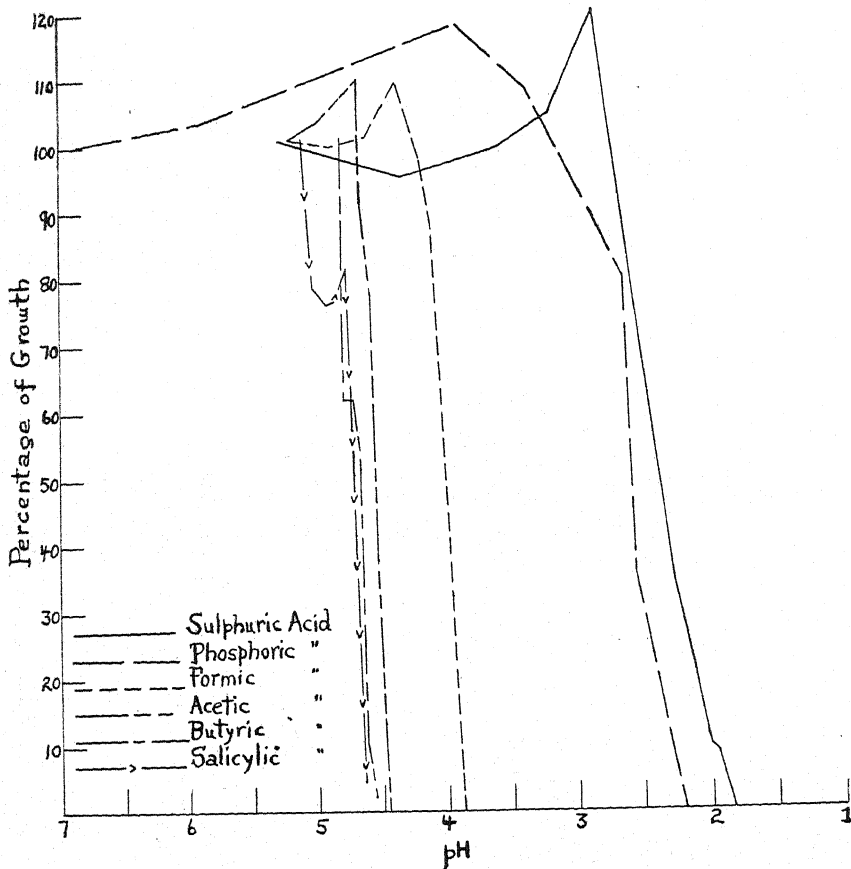
The percentages of relative growth values for the fungus in the sodium-salt cultures corresponding to the acid cultures in which the least amount of growth was obtained for a particular series were found to be as follows: sodium sulfate, 94.2 percent; sodium hydrogen phosphate, 82.2 percent; sodium formate, 100 percent; sodium acetate, 100 percent; sodium butyrate, 3.6 percent and 4.7 percent; and sodium salicylate, 72.9 percent. The toxic effect of sodium butyrate was marked; in both series run with this salt (tables 13, 15), 2.5 cc. of N/10 salt allowing only 3.4 percent–3.6 percent growth.

#### DISCUSSION

A comparison of the order of toxicity of equinormal solutions of a series of acids should give some indication of the importance of the hydrogen ion as a factor in toxicity. If the hydrogen ion is the chief factor or sole factor of toxicity of the acids, then those acids which are more highly dissociated should be relatively more toxic. Owing to the widely different concentrations necessary under the conditions of these experiments, a strict comparison of percentage growth in equinormal solutions is impossible. For example, the solubility factor of salicylic acid made advisable the use of a higher dilution (N/100) of this acid. At the same time, the final volume of the culture medium in all culture flasks was limited to 50 cc., 25 cc. of which was basal nutrient solution. At N/32, sulfuric and phosphoric acids were practically equal in value (102 percent–103 percent) while at N/16, sulfuric acid gave 10 percent and phosphoric acid over 110 percent growth. At N/200, formic acid gave 99+ percent growth, acetic acid 105 percent in one series, butyric acid 3.2 percent–3.4 percent, and salicylic acid 4.8 percent. With the concentrations used greater than N/125, acetic acid becomes more toxic than formic acid. With butyric acid at N/800, the percentage growth was approximately between 50 percent and 60 percent, while with salicylic acid it was 78 percent; with butyric acid N/333, 53.1 percent in one case (table 11), and 4.5 percent (table 14) in another; with salicylic acid N/333, there was 75.9 percent growth. It may be seen from figure 3 that butyric acid is more toxic than salicylic acid at all the comparable concentrations used.

The order of toxicity found in these experiments for equinormal solutions is as follows: at N/20 and at higher concentrations, sulfuric > phosphoric; at N/200, butyric > salicylic > formic > acetic; with N/83 and higher concentrations, acetic > formic. The general order found when based on molecular concentration is butyric > salicylic > acetic > formic > sulfuric > phosphoric (text fig. 3). Clark (2) has shown that in equinormal

solutions acetic acid is much more toxic than sulfuric and hydrochloric acids to *Botrytis* and certain other fungi. Collett (4) found the general order for *Paramoecium* in equinormal solutions to be hydrochloric > salicylic > formic > acetic and butyric acids. Kahlenberg and True (9) in their work on *Lupinus* seedlings showed the order of toxicity for equinormal solutions to be hydrochloric > salicylic > formic > butyric. Bodine (1) gives the general order of toxicity for mosquito larva: salicylic > butyric > acetic. The results here reported differ from those of the three last-



TEXT FIG. 2. Percentage of growth of *S. cinerea* at various hydrogen-ion concentrations with various acids.

mentioned workers in that sulfuric acid is less toxic than the fatty acids, and that butyric acid is more toxic than either salicylic or formic. The above-named writers, except Clark, used very weakly buffered solutions where additions of equal amounts of different acids must have produced far different degrees of acidity. In these experiments, where a highly buffered

medium has been used, the buffer action of the solution would reduce the relative differences in the actual acidity produced by highly and less highly dissociated acids. Hence it is probable that the buffer action of the solution is a factor which has changed the relative position of the mineral acids in the series. Increasing the length of exposure to mineral acids because of their slow penetration into a cell should tend, if it causes any change, to render the acid relatively more toxic than the acids which enter more readily. If this assumption is true, it would seem to indicate that the toxicity of the mineral acids has not been exaggerated in these experiments where a longer time limit than in the investigation of others was chosen as a criterion.

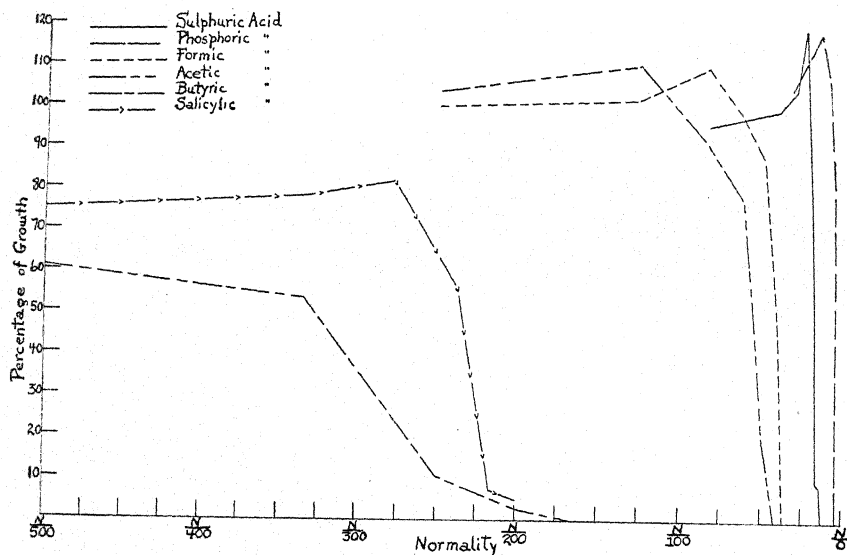
The results show that the hydrogen ion is not the only factor or the chief factor to be considered in the toxicity of salicylic and the fatty acids to *Sclerotinia cinerea*, since the order of toxicity does not parallel the order of dissociation for these acids.

It is of interest to notice that the relative toxicities of salicylic and the fatty acids in equinormal solutions for *S. cinerea* are in accord with the order that would be expected from the standpoint that the more highly developed the non-polar part of the molecule, the more readily primary penetration occurs (7). The mineral acids used also are in the position in the series that we should expect when we view these acids as only entering the cell as a result of the injury to the cell by the H ions, higher concentrations therefore being necessary to produce toxic action.

Another source of evidence of the toxicity of the hydrogen ion is a comparison of the percentage of growths of *Sclerotinia cinerea* obtained with the acids used at the same hydrogen-ion concentration. If the hydrogen ion is the only toxic factor, then it is clear that at the same pH the acids should be equally toxic, and the percentage growths should be about equal. It may easily be seen from figure 2 that this is not the case. At pH 4.0, there is about 45 percent of the growth of the control with formic acid, 96 percent with sulfuric acid, and 116 percent with phosphoric acid; and at pH 4.70, with salicylic acid 59 percent, with butyric acid 61 percent, with sulfuric acid 96 percent, with formic acid 100 percent, with acetic acid 108 percent, and with phosphoric acid 111 percent. As may be seen, the curves for the percentage growth of the different acids cross each other at certain points (fig. 2). The curves for sulfuric and phosphoric acids are alike in many respects—approximately 80 percent growth being obtained at pH 2.6 and pH 2.68 respectively. The general order of toxicity at pH 4.70 is salicylic > butyric > sulfuric > formic > acetic > phosphoric, while with a little higher hydrogen-ion concentration (pH 4.5), acetic acid is far more toxic than sulfuric acid, and at pH 4.4 and above formic acid is also more toxic.

It may be seen that in the comparison of solutions of equal pH, salicylic and the fatty acids keep their position as being more toxic than the inorganic acids. Collett (4), in describing her experiments on *Paramoecium*, states that in solutions of equal pH acetic and butyric acids become equal in toxic-

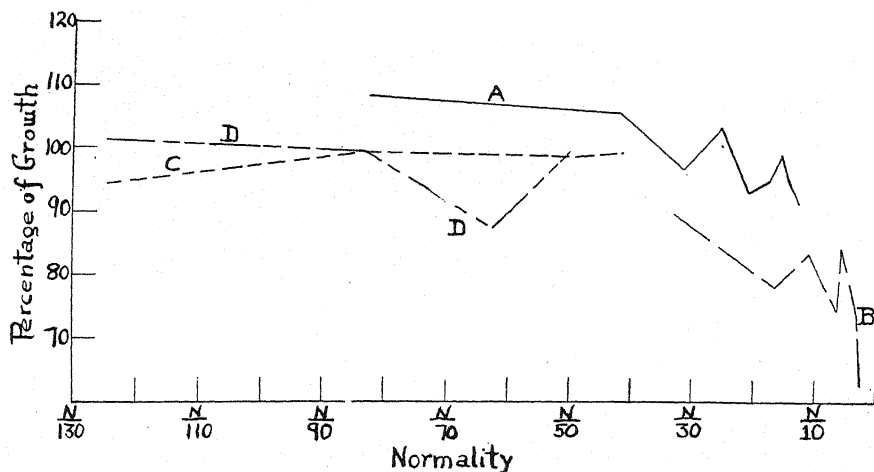
ity to the cyclic acids and that the dibasic and hydroxy acids together with hydrochloric acid become least toxic of all. The fact that fatty acids are more effective physiologically than hydrochloric acid of the same H-ion concentration has been observed by certain workers. For example, Loeb (11) found in his experiments on membrane-formation in sea-urchin eggs that  $\text{CO}_2$  and the fatty acids were efficient and that strong mineral acids were inefficient. He explained the phenomena on the basis of the greater diffusibility of the non-dissociated acid molecule through the cell wall. It is striking to note that the order of toxicity of the acids in the complex nutrient solution used in these experiments is of the same order as that found in the above-mentioned investigations. It is thus safe to assume that these results indicate the toxicity of the acid used rather than that of any uncertain factor which may have been introduced by the complex solution. Again, if the effect on growth is proportional to the ability of the acid to penetrate the cell and to produce some physiological upset, we have indirect evidence that we should expect this order of toxicity from the work on permeability (7).



TEXT FIG. 3. Percentage of growth of *S. cinerea* at various normalities of the acids used.

The very fact that with salicylic and the fatty acids no growth occurs under the conditions of the experiment at a higher hydrogen-ion concentration than pH 3.99 shows that the hydrogen ion can not be the chief factor in toxicity, whereas in the case of sulfuric acid growth was found at pH 1.95 and with phosphoric acid at pH 2.2. Furthermore, acetic-acid cultures containing only the nutrient solution with 1 cc. of normal acetic acid and

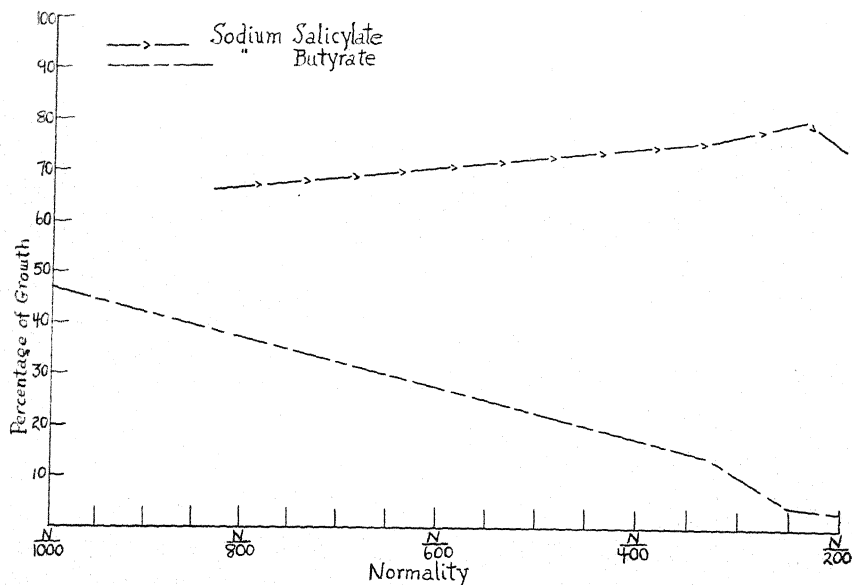
with no sodium hydroxid added failed to give growth although the pH was only 3.85. Likewise, another series of acetic-acid cultures, in which the acidity of the nutrient solution was lowered by the addition of sodium hydroxid to pH 5.65 (table 10), failed to give growth at pH 4.69 with 1.2 cc. normal acetic acid added. Butyric-acid cultures likewise, when the original nutrient solution was at pH 4.80 showed a hydrogen-ion concentration at growth limit of about pH 4.5, while, when the original hydrogen-ion concentration of the nutrient solution was pH 5.2, growth ceased at pH 4.79. In the cases of salicylic and the fatty acids used, it seems that the original pH of the medium and the amount of the acid added, and not the hydrogen-ion concentration alone, determined the limits of growth of *Sclerotinia cinerea*.



TEXT FIG. 4. Percentage of growth of *S. cinerea* at various normalities of (A) sodium sulfate, (B) sodium dihydrogen phosphate, (C) sodium formate, (D) sodium acetate.

Since it is evident that, in the cases of the fatty acids used and of salicylic acid, the hydrogen ion is not the sole factor, it is obvious that the toxicity is due to other causes. It is impossible to show directly that either the anion or the undissociated molecule of the added acids is the toxic agent until the combinations formed in the complex highly buffered nutrient solution by the added acids and their percentage dissociation can be determined. However, a comparison of the salt solutions in equinormal concentrations with their acids shows certain tendencies. Figures 4 and 5 show the kind of curves obtained for the various sodium salts used. Series were run for each salt which duplicated the steps of the series of the corresponding acids. By a comparison of the percentage growth curves, it may be seen that the salts of the acids are in general far less toxic than the acids themselves. Sodium butyrate alone shows a tendency to approach the toxicity of its acid. In one series (table 13), for example, sodium butyrate N/333 gave a growth of 14.2 percent, while butyric acid N/333 gave a value of 53.1 per-

cent. In the same series, sodium butyrate N/250 gave 4.4 percent growth while butyric acid N/250 allowed 10.6 percent. In another series, sodium butyrate N/333 gave a growth value of 40.7 percent and at N/250 only 4.5 percent. Butyric acid in the latter series at N/333 gave a value of 4.5 percent and at N/250 no growth. While it is not possible to state the percentage dissociation of the salt solutions, it is known that there is a



TEXT FIG. 5. Percentage of growth of *Sclerotinia cinerea* at various normalities of sodium butyrate and sodium salicylate.

tendency for the salts of the acids to dissociate more highly than the corresponding acids. Since we know that the sodium cation is practically harmless, and if we assume higher dissociation of the salt than of the butyric acid, then it appears that the toxicity of butyric acid is in large measure due to the anion and the undissociated molecule. However, in the case of butyric acid, the relatively greater toxicity of the anion is apparent when compared with formic, acetic, and salicylic acids.

In all other cases with the higher concentrations used, the salt is much less toxic than the corresponding acid. Sodium dihydrogen phosphate gives between 70 percent and 80 percent growth at the limiting concentration for phosphoric acid, while sodium sulfate gives 94.2 percent. It appears that in the case of these mineral acids, since ionization is high, the main toxicity factor is the hydrogen ion. This agrees with the work of True (15) and Winslow and Lochridge (17).

With respect to formic, acetic, and salicylic acids, if no other toxic compounds are formed and if the salt dissociates far more than its acid in the

nutrient solution used, then the undissociated molecule may be suspected as the principal toxic factor. The anion appears to be relatively nontoxic. This is in accord with the results of Collett (4), Kahlenberg and True (9), True (15), and Winslow and Lochridge (17).

#### SUMMARY

1. The addition of sodium hydroxid is practically harmless in changing the pH from 3.8 or 4.0 to 5.2 or slightly higher.

2. A slight amount of acidity is beneficial for growth, the best results with sulfuric and phosphoric acids being obtained between pH 2.85 and pH 3.9.

3. There is a fairly narrow zone on the acid side which limits growth for each acid used—the percentage growths falling in an almost perpendicular line (fig. 2).

4. The general order of toxicity for solutions under the conditions of these experiments at pH 4.70 is salicylic > butyric > sulfuric > formic > acetic > phosphoric, while at pH 4.50, acetic is more toxic than sulfuric, and at pH 4.4 formic is also more toxic than sulfuric.

5. A comparison of the toxicity of the acids on a basis of normality gives the general order: butyric > salicylic > acetic > formic > sulfuric > phosphoric. This is the order that would be expected from the comparative ease of penetration of the acids into the living cell as has been shown in other investigations.

6. There is indication that the anion of butyric acid may be relatively toxic.

7. The toxicity of the fatty acids used and of salicylic acid is probably due chiefly to the undissociated molecules, with the hydrogen ion playing a secondary rôle.

8. On the other hand, the hydrogen ion is the principal factor of toxicity in the case of the mineral acids used.

In conclusion, these results show that the hydrogen ion is not always the chief factor of toxicity in the effect of various acids upon the germination and growth of fungous spores.

The author wishes to express to Dr. Charles H. Arndt his appreciation and thanks for many valuable suggestions and constant interest throughout the progress of the work.

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## CHROMOSOME STRUCTURE AND ITS RELATION TO THE CHROMOSOME CYCLE

### I. SOMATIC MITOSES IN *TRADESCANTIA PILOSA*

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(Received for publication April 21, 1925)

While studying the structure of the chromosomes of *Tradescantia pilosa* Lehm. during the maturation phases, details of chromatin organization and arrangement were encountered which required for their elucidation a critical analysis of the somatic mitoses. One of the most perplexing problems concerned the almost constant arrangement of the chromatin in the form of spiral threads. There is nothing new in the discovery of such spiral bands in plant chromosomes, but considerable controversy has arisen as to their possible function and relation to the chromosome cycle. Having observed the chromatic spiral threads in the chromosomes of all the phases of the first maturation division, the question naturally arose as to whether such organization was restricted to meiosis. For an answer it was necessary to consider both the somatic and premeiotic nuclei, which were alike in most details. The relative ease with which root tips could be obtained made it advantageous to work with them. Figures of premeiotic cells have been included only to corroborate certain features. Discussion of the meiotic phases will be reserved for a subsequent publication.

The divergence of the cytological observations so often presented by different workers on the same cells is enough to show that any effort to discern the smaller visible elements of chromosomes must require the application of a variety of fixatives in lengthy series, so that the different appearances produced may be properly evaluated. This is particularly true of *Tradescantia*, which has been noted for the difficulties it presents in obtaining adequate fixation. Referring to *T. virginiana*, Strasburger (1900) remarked, "Zwar lassen sich die karyokinetischen Bilder in diesen Pollenmutterzellen im Allgemeinen nicht gut fixiren." Farmer and Shove (1905) stated that similar difficulties were encountered. Sharp (1920), studying the somatic mitoses of the same species, commented on the inferiority of his preparations of this material for late prophase stages as compared with

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those of *Vicia*. However, he regarded *Tradescantia* as superior material for analysis of the critical stages of the telophases and early prophase.

The tediousness of the paraffin method has been an important factor in curtailing efforts to obtain critical fixation. For eliminating that technique I am particularly indebted to the smear method recently described by Taylor (1924). This allowed the application of a lengthy series of fixatives in much less time than would have been required by the paraffin or the celloidin method. Although designed for the study of the developing microsporocytes, the smears disclosed dividing tapetal cells of the microsporangium in sufficient quantity to give some idea of the value of the fixative for somatic mitoses. Such values were only an index, however, and the fixatives usually required certain alterations of the proportions of their constituent chemicals before being entirely adaptable to the paraffin method. This is in harmony with McClung's (1918) comment that a fixative which produces good smear preparations may give atrocious results with even a reasonable mass of tissue.

No one formula can be detailed as that of a universal fixative. During two growing seasons and a portion of a third the cells of this plant were fixed in various modifications of a number of types of killing agents, with the apparent result that an excellent preservative of the elements during one phase often proved quite destructive to those elements during another phase. The best fixations were obtained with chrom-osmic-acetic acid combinations, to which such adjuvants as lactose, maltose, and urea were added.

Staining of the preparations used for the following study was done by the iron-alum haematoxylin method. For purposes of viewing the details of those stages wherein the chromosomes have the greater affinity for the dye, such as the metaphases and anaphases, it was found necessary, as shown by Lee (1920) and Martens (1922), to curtail considerably the period of staining. I found that a period of staining of one hour or less in a one fourth to one half percent solution of haematoxylin, following mordanting for an equal time in two and one half percent iron alum, gave a density of stain which could readily be differentiated. The alternative method, of staining six to twelve hours in a one half percent solution and differentiating for a sufficient period of time to bring out the desired structure, more often resulted in a brownish black or muddy color than in the desired clarity.

I am greatly indebted to Dr. W. R. Taylor, under whose direction this problem was conducted, and to Dr. E. Eleanor Carothers, Prof. Clarence E. McClung, and Dr. William Seifriz for valuable suggestions and criticisms.

#### HISTORICAL

Study of chromosome structure in members of the genus *Tradescantia* dates back to the work of Baranetzky (1880). His investigations concerned

*T. virginica*, *T. pilosa*, *T. subaspera*, *T. discolor* (*Rhoeo discolor*), and *T. zebrina*. Because he did not like the fixation appearances of material preserved in alcohol, Baranetzky confined his observations to living cells. Anthers were crushed under the cover glass and the extruded cells were studied in spring or in salt water. Although Baranetzky described the nuclear thread as having the appearance of alternating discs of darker and lighter material, this was the aberrant condition. A more critical interpretation found the thread composed of two substances, with the denser confined to the superficial layer and in the form of a spiral band, which view was verified not only by the observation of the coiled but also by that of the uncoiled thread (Baranetzky's figs. 41 *a, b, c, d, f, h*). Baranetzky realized that such definite structure was perhaps indicative of a fundamental functional relationship: "Ob der dichten Spiralfaser dabei wirklich irgend eine mechanische Rolle zukommt, mögen spätere Untersuchungen entscheiden."

Strasburger (1882) did little work on living objects, but drew his conclusions from fixed material. His observations on pollen mother cells of *T. subaspera* revealed a nuclear thread composed of alternating discs of darker material and lighter "Zwischensubstanz." To these he applied the terms "Nucleo-mikrosomensubstanz" and "Nucleo-hyaloplasma." He admitted his inability to see a tightly wound spiral and reported unsuccessful efforts to confirm Baranetzky's experiment of uncoiling such a spiral. He gave no figures of *Tradescantia* in this paper, but his figure 73 *b* of *Fritillaria imperialis* illustrates the type of chromosome structure described.

Miyake (1905) also limited his observations to the meiotic phases. He noted that, so long as study was restricted to fixed material, absolute certainty could not be attached to the interpretations. His figure 148 of a metaphase of the first maturation division gives some indication of internal chromosome structure, although it is illustrative of chromomeres or "Körnige Struktur" and not of a spiral thread.

Farmer and Shove (1905) described both somatic and meiotic phases in *T. virginica*. The arrangement of the chromatin within the linin filament of the early somatic prophase was of "the intermittent character to which the appearance of alternate stainable and non-stainable discs is due." The chromatin discs or granules were later presumed to split in order to produce the longitudinal fission of the chromosome.

Van Herwerden (1910), verifying Baranetzky's observations, studied *T. zebrina* by expressing the nuclear thread from the cell into dilute acetic acid and methyl green. She noted that it would be difficult to describe these threads as other than spiral, although their size was not so favorable for study as in the case of the *Chironomus* larva, a consideration of which formed the bulk of the paper. She thought the spiral is but a temporary phase of the mitotic division in *Tradescantia*, whereas in *Chironomus* it is a persistent structure.

A more recent contribution, restricted entirely to chromosome structure, particularly of *T. virginiana*, is that of Sands (1923). Somatic as well as meiotic chromosomes were described and figured. Sands employed primarily Belling's aceto-carmin method, although fixed material and pollen mother cells teased out on a slide into three-percent cane sugar solution were also used. In the aceto-carmin preparations, "the structure came out with the first swelling, somewhat as does the image on a photographic plate, without any perceptible change either in the form or in the position of the elements from that seen in the living condition." The chromosome was pictured as a linin cylinder within which the chromatin material is peripherally disposed. The latter exists as chromomeres, and these are variable in size, shape, and number. Indications that they may assume the appearance of a spiral thread were acknowledged by Sands when he said that "sometimes they show traces of a spiral arrangement (figs. 17, 18, Pl. XXX)." <sup>1</sup> The linin substratum was regarded as of jelly-like consistency, and in it the chromatin was considered to be imbedded. The colloidal structure was considered as primarily a two-phase system with the linin representing the continuous and the chromatin the disperse phase.

The most critical analysis of somatic mitoses was made by Sharp (1920) on root tips of *T. virginiana*. He described a telophasic vacuolation transforming the chromosomes into alveolar-reticulate structures. In the following prophase a single chromatic thread appears to be formed from each of the chromosomes. The splitting of that thread was described as occurring by means of the formation of a series of vacuoles, most of which were regarded as new formations and not as structures persisting from the preceding telophases. The true splitting of the chromosomes was therefore considered to be a prophase development and not to be traced back to the telophasic vacuolation.

## OBSERVATIONS

### Anaphases

At the inception of the anaphases the chromosomes arch towards the poles, forming long V's, the median constriction of each serving as the point of spindle-fiber attachment. The median constriction is a zone of variable width, sometimes recognizable only as a narrow slit (Pl. VI, fig. 1), at other times appearing as a conspicuous area between the chromosome arms (fig. 2). Under proper differentiation of stain it appears as a grayish-black zone of less diameter than the denser black arms. Robertson (1916) has applied the term "achromatic bridges" to such points of spindle-fiber attachment found in V-shaped chromosomes. This designation is applicable to the anaphase chromosomes of *T. pilosa*, since they present evidence of differentiation into two dissimilar substances to which the terms *chromatic*

<sup>1</sup> These are photomicrographs of pollen mother cells of *Rhoeo discolor*, in the metaphases of the first meiotic division. In these cells I have seen the same type of spiral structure to be described for *Tradescantia pilosa*.

and *achromatic* may be applied. Martens (1922) has observed a similar differentiation within the chromosomes of *Paris quadrifolia*, but he notes and figures the achromatic material as showing some affinity for the stain. This is equally true of my preparations. The terms *achromatic* and *chromatic* therefore become merely relative and replaceable by the terms *oxychromatin* and *basichromatin*, with which they are here regarded as synonymous. Where differentiation of the stain is not sufficient, the achromatic and chromatic materials present such a homogeneity of color that the constriction, if recognizable at all, is distinguished only by its comparative slenderness. Apparently the two systems of the chromosome have at anaphase a more nearly equal affinity for the stains than during some of the other phases. This fact necessitated the method of staining described above. Using such a method, the chromosome appears no longer a homogeneous unit. Its end view (fig. 3) suggests a hollow ring with a narrow lumen, showing that the more densely staining material is peripherally disposed. The lumen is actually a space between the ends of two chromatic threads which can be seen as intertwined spirals when the chromosome is viewed laterally (fig. 4). Since these spirals are homologous with the *chromonema* of Vejdovský (1912), as will be demonstrated later, it would perhaps be well to recognize the term *chromonematic substance* used by Martens as synonymous with the term *chromatic substance*. Between the spiral threads can be recognized the lighter-colored achromatic material. Such observations suggest that the chromosome is essentially a long rod of achromatic material, of which the median constriction is a part and within which is peripherally disposed the chromonematic material. Further verification of this composite structure is offered by such a chromosome as that shown in figure 5. This was taken from a cell several layers below the surface of the root, where penetration of the fixative was hindered sufficiently to allow of distortion and breaking of the chromonemata. Accordingly, the aspect figured must not be regarded as a criterion of delicate structure perfectly preserved, but it is valuable in demonstrating the existence of the two systems composing the chromosome. There is also the suggestion of a limiting membrane to this chromosome, a condition exaggerated quite beyond the normal, in which condition membranes can not be seen to occur. But failure to see them does not offset the probability of their existence, as was suggested by Seifriz (1921).

That the chromatic substance really exists at this stage in the form of spiral threads is verified by the following evidence: (a) Even when the stain is too deep to permit a visible differentiation of the substances of the chromosomes, the undulating contour and the resultant moniliform appearance suggest a definite internal organization. There can be no question that such an outline would be formed by two intertwined spiral threads. Where the threads appear to cross, that is, where they are lying one above the other, the chromosome appears narrower. In the gradual transition

from that region to the one where the threads appear to lie side by side, the chromosome outline gradually broadens, to constrict once more as the threads appear to recede from the common plane. Such a moniliform aspect can be observed in any lateral view (figs. 6, 12). Bendings, torsions, and foreshortening naturally do much to offset the maximum diagrammatic appearance. (b) The chromosome often shows a terminal indentation or notch which is highly suggestive of the beginning of a longitudinal split. Projecting slightly on each side of the notch is the end of a thread (figs. 4, 6, 7). (c) Torn ends of chromosomes occasionally show two projecting threads. Sometimes their angle of divergence is great enough to offset any doubt as to the duality of structure involved (fig. 8). (d) When the chromosome can be visibly analyzed, the chromonemata can be traced. In staining for these structures, the achromatin colors slightly or not at all, so that where the chromatin can be most clearly observed the achromatic material is often indistinguishable. Figures 4, 9, 10, and 11 demonstrate the continuity of the chromonemata. There is apparently no preciseness to the pitch of the spiral in different chromosomes or even in the same one. Loose coils seem more abundant than tight ones, and this is probably because of the extreme length of the chromosomes, the origin of which can be traced back to their behavior during the prophase. It can be determined that in some cases the helix is dextrorsal and in other cases sinistrorsal. I could not determine whether the number of chromosomes representing each of these types was equal or not. It is clear that, since the cleft between the chromonemata here follows a spiral and not the long axis of the chromosome, it can safely be accepted as an actual rift and not an apparent one.

Satellites occasionally are seen in these anaphase cells. The satellite is a most delicate terminal appendage, seemingly imbedded in the apex of a conical projection of achromatic material (figs. 14, 15). From the limited number of specimens clearly illustrating these structures it appears as if but a single satellite exists on one arm of one of the chromosomes moving toward a pole (fig. 6).

As migration to the poles proceeds (fig. 12), it is usually impossible to detect spindle fibers, owing perhaps to the type of fixative and stain employed. A gradual shortening and thickening of the chromosome arms occurs. This is possibly accomplished by the tightening of the spirals, for many of these chromosomes give indications of more closely wound threads than during the earlier stages (fig. 10). Many of the typical V's are altered by the bending of one or both of the arms. Occasionally it seems as if a certain amount of untwisting of the chromosome occurs, which results in an extension of at least part of the spiral (fig. 16). This chromosome is particularly interesting, not only because of the verification of the longitudinal split between the halves, but also because of the suggestion of chromomeres. These can be noted as more darkly staining zones of

greater diameter than the rest of the thread, so that they stand out as bulges or swellings. The fact that they as well as the chromonemata are paired, seems to indicate that on further investigation the type of organization here involved might prove to be as precise as that detailed by Wenrich (1916) for certain chromosomes of *Phrynotettix*.

Continued shortening occurs until the chromosomes approximate at the poles. From the upper group of figure 18 it would appear as though clumping had occurred to the extent of obliterating the boundaries of the component individuals. It seems to me, however, that this grouping is either an artifact or an optical effect due to the mass of the material and the density of the stain. This premise is strengthened by the appearance of the lower group of the same cell and by figure 17. In these cases the members of the complex can be recognized as maintaining their individuality. More specific evidence is offered by the structure within the chromosome. Even when clumping does seem to occur, a projecting member or a portion of one may reveal the chromonemata. Thus, chromosome *a* of figure 18, detailed in figure 19, shows a tightly wound coil, suggesting that the shortening may in part be accomplished by the tightening. Projecting ends of two chromosome arms are also shown in figure 20. If an isolated individual is considered, as in figure 21, the notched ends and twisted threads indicate that the continuity of the chromonemata has not been effaced. Neither is there any suggestion that the chromatic and achromatic systems have lost their identities.

The fibers between the two groups of chromosomes are now pronounced, and the cell plate is beginning to differentiate across the fibers at approximately a median position.

### Telophases

There is presented, to complicate the analysis of the telophase activity, a series of changes concerned with the transition of the chromophilous anaphase chromosomes into the delicate threads of the late telophases and the apparent reticulum of the interphases. Very early in this process a limiting membrane is established around the nucleus, separating its contents from the cytoplasm. This membrane can be seen surrounding the occasional projecting chromosome before it can be traced around the entire nucleus. The suggestion is thereby offered that this limiting layer is but a more pronounced expression of the membrane which earlier marked the boundary between the achromatic system of the anaphase chromosome and the cytoplasm.

The question arises as to the disposition of the achromatic material. In the nuclei examined, the karyolymph, at this early stage of its existence as a distinct substance within the newly formed nucleus, shows more affinity for the stains than at later times. A consideration of such a nucleus as that shown in figure 23 shows that the contrast between the chromatic material and the karyolymph is hardly more decided than the contrast



between the two systems of the anaphase chromosomes. The indication of morphologic continuity within the isolated chromosome, as well as the apparent continuity of staining quality, suggests that the karyolymph is a direct product arising from the achromatic material of the chromosomes composing the daughter nucleus. When this material abuts on the cytoplasm a membrane is formed. Where chromosomes are in contact no need for any such membrane arises, and the karyolymph becomes a continuous colloidal system bearing the chromonematic skeletons of the members of the group.

In addition to showing the densely staining karyolymph, figure 23 is valuable in demonstrating the chromonemata during these early telophase changes. It will be noticed that in chromosome *a* the membrane is separated by a narrow zone of karyolymph from the chromonemata and that anastomosing processes run out from the spiral threads to the membrane. Where the contrast of staining of anastomoses and chromonemata is sufficient, the spiral threads can readily be traced and the direction of the twist diagnosed as in this case. But when the contrast is not so decided, as at *b*, the appearance of a reticulum is offered. A sac, such as this, into which the ends of two or more chromosome arms are projecting, offers, by reason of the abundant anastomosing, much difficulty to critical analysis.

With the progress of the reorganization of the daughter nucleus, the chromonemata become more pronounced through the diminution of the affinity of the karyolymph for the color (fig. 24). Delicate attenuations are seen to arise from the slightly fuzzy surface of the chromonemata and pass to connect adjacent chromosomes or even parallel threads of the same one. My observations do not support the contention that these inosculation are adhering portions of chromosomes once pressed closely together. They are outgrowths of the highly active, mobile chromatic threads.

There is an indication in figure 24 as to the origin of the nucleoli. Small, deeply staining granules are shown. They are seen at the ends of the chromosomes and always maintain some connection with them. Larger nucleoli are also obvious, often where the ends of two or more chromosomes are contiguous. Because of their position the smaller granules recall the "polar granules" described for *Phrynotettix* by Miss Pinney (1908) and Wenrich (1916). The larger nucleoli are apparently compound structures or multiples formed by the union of a series of the smaller bodies. Whether these nucleoli represent unaltered portions of anaphase chromosomes or reservoirs of chromatic material is difficult to determine by these stages. The gradual diminution of the diameter of chromonemata induces the opinion that the latter may be the probable situation. Furthermore, I have not succeeded in distinguishing the detail within these nucleoli which would probably be seen if they contained the two systems found within the anaphase chromosomes. The attachment maintained between nucleolus and chromosome is shown in figure 26, Plate VII.

One of the most diagrammatic representations of the chromonemata and of the polarity of the chromosomes during the telophases is furnished by the nucleus shown in figure 27. It is from a premeiotic cell of a very young microsporangium and is inserted here to illustrate the precise nature of the arrangement of the threads and their relation to the nucleoli.

As the telophases advance, the anastomoses become more pronounced and the outlines of the chromosomes less conspicuous (fig. 28). However, little difficulty is encountered in following the course of the spiral threads, and the hollow-ring appearance of the chromosomes in end views demonstrates the persistence of the cylindric type of structure. Larger nucleoli appear in these nuclei, certainly fusion products of the smaller ones of earlier stages, as is shown by the connecting bridges of figure 29.

A further stage of the development of the anastomoses is seen in figure 30. The chromosome at *a* presents the appearance of a pair of regularly interlaced zigzag threads. Darker spots resembling granules are seen at the points of crossing and bending of the threads. They represent only doubleness or greater amount of thread surface, and not chromomeres. If the latter exist at this time as morphologic units, they are not visible in these preparations.

The chromonemata undergo a gradual diminution of their diameter during the telophase changes. Apparently this diminution is beginning in figure 25, Plate VI, and is quite pronounced in figures 30 and 31, Plate VII.

Up to the time of the formation of the reticulum, in those nuclei in which it is formed, the limits of the constituent chromosomes can be traced with a gradually diminishing degree of preciseness. This is due, not to the loss of morphologic continuity, but to the pronounced inter-chromosomal inosculation. It is also apparent that the nucleus is assuming a more nearly spherical form, a condition which has arisen from the irregularly shaped, many-lobed creation of the early telophase. In the transformation a lengthening of some of the chromosomes must occur, accompanied by much bending and twisting.

### Interphases

In the actively dividing meristematic cells mitoses often follow with sufficient rapidity to prevent the intervention of a definite period of rest. This fact is demonstrated by the sparsity of the characteristic resting nuclei as compared with their abundance in the less active or in the older portions of the root. The lobed appearance of many of the prophase nuclei is also suggestive of the shape assumed during the telophases and indicates the failure to proceed to the "resting stage." In a nucleus which has progressed through the telophase changes sufficiently to present a somewhat uniform reticulum, distinction can be made between the constituent chromosomes only occasionally and with difficulty. There is reason to believe that the identity of the chromosomes has not been lost but is only concealed in the reticulum. For example, at *a*, figure 32, the intertwined threads can

be traced. Less favorable examples are offered by other parts of the nucleus where the like affinity of the anastomoses and the chromonemata for the stain prohibit accurate determination. Where the threads appear to cross or where they bend the effect of more deeply staining granules is produced, but these appearances must not be interpreted as chromatic granules supported on a linin network. The linin or achromatic material, as demonstrated by the changes of the telophases, is now to be considered as a part of the karyolymph and continuous with it. Certainly the anastomoses are not to be regarded as a part of the achromatic material, for they, by their origin and persistence, indicate their relation to the chromatin. Larger nucleoli as well as smaller ones are present at this time and usually can be seen to maintain connection by delicate threads with the reticulum and not to lie free in the karyolymph entirely surrounded by a "perinucleolar space."

### Prophases

Conceding that only some of the telophase nuclei pass to the "resting stage," it follows that the prophase begins in one case with the chromosome outlines visible and in the other case with them obscured. In those nuclei which have taken on the reticulate type of structure, the first indications of the reappearance of the chromosomes are furnished by a partial withdrawal of the anastomoses (fig. 33). Accordingly, clear spaces come to exist at the places which will mark the boundaries of the chromosomes. Narrow at first, these spaces enlarge as the anastomoses are withdrawn more and more, and the chromosomes become prominent. A consideration of figure 33 reveals that in some portions of the nucleus a definite duality of intertwined parallel threads is visible. It is extremely difficult to understand just why the space between the two threads has become so narrowed since the telophases, unless it is remembered that in the formation of the spherical nucleus lengthening and other movements of the chromosomes have taken place. Approximation of parallel filaments was suggested in figures 26, 27, and 29, but the maximum expression of that affinity is found in these prophase nuclei.

Conspicuousness of the chromosome is enhanced by the additional withdrawal of anastomoses (fig. 34). Here the chromosomes are seen stretched across the nucleus, those nearer the center somewhat more extended than the peripheral ones. The latter seem to be more restricted in their activity as a result of their positions. No differentiation into the chromatic and achromatic materials is visible within each chromosome, so that the chromonemata contrast with the faintly coloring karyolymph. There is no question as to the spiral structure. Ability to trace the coils is sometimes possible; again the *u* or zigzag suggests the type of structure involved. As in the earlier stages, the question of singleness or duality of these filaments is introduced. It will be noted from figure 34 that, although some of the threads do appear as single, there are many proofs of

duality of elements. Numerous interlaced double spirals similar to those observed in the telophases are present (chromosome *a*). Most of these are nearer the periphery than the center of the nucleus and suggest chromosomes that have not undergone a stretching or lengthening. In many of the stretched chromosomes two filaments are observable as delicate parallel threads. That the conspicuous double spiral with the bands widely separated can grade over to the type with the threads almost or quite in contact is demonstrated by figure 35, taken from another nucleus. Chromosomes at this time still maintain connection with the nucleoli (fig. 36). The presence of crypts, crevices, and irregularities in the surface of the latter indicates a gradual transformation of a portion of the contained material.

Nuclei which did not continue to a complete reticulum in the telophases show clearly in the early prophases the limits of the included chromosomes (fig. 37), although some anastomosing is apparent. Continuity of the chromonemata can be traced in some of the chromosomes although it is obscured in others. At a slightly later stage (fig. 38), when the anastomoses are more delicate and not so pronounced, the double spiral threads are discernible. The hollow-ring appearance of the end views is also in evidence (fig. 39). Certain nucleoli, such as the larger one of figure 38, demonstrate their composite nature. The connecting bridges suggest a fragmentation of the larger masses into smaller units (see also fig. 40).

Stretching of the coils is now proceeding rapidly and is accompanied by a certain amount of untwisting. Each of the chromosomes appears as in figure 41, a pair of slender intertwined threads. From these, very delicate pseudopodium-like processes extend out into the karyolymph and across it to the neighboring chromosomes. Such projections are most characteristic surface developments of the chromonemata during the early prophase stages. The threads connecting the chromosomes to the nucleoli are also most pronounced. In the case shown in figure 42 it seems as though the material of the nucleolus were being dissolved, perhaps to be of service in the growth of the chromonemata.

Subsequent development is concerned with the growth of the threads and their uncoiling (fig. 44). The chromonemata at this time are very fuzzy or woolly, numerous processes running out from their surfaces. Some of these traverse the spaces between the parallel threads and appear to connect them. A pair of threads so bridged by anastomoses may at times appear as a single vacuolated band. But it must not be forgotten that the processes extending from the chromonemata are as pronounced or even more pronounced across the inter-chromosomal distances of the karyolymph as across the inter-chromonematic spaces.

As thickening of the threads occurs, it is certain that a shortening and unwinding is also occurring (fig. 45, Pl. VIII). In this nucleus the chromosome stretched across the diameter is already uncoiled, the parallel elements lying side by side, while the more peripherally disposed members still show

the intertwined halves. The anastomoses are decidedly conspicuous, both between the halves of the chromosomes and between the adjacent chromosomes. At this time it becomes apparent that in some of the threads there is an internal distinction between more darkly staining regions and the lighter connecting areas. At a slightly later stage (fig. 46), these chromomere-like regions stand out as more pronounced swellings which bulge beyond the diameter of the thread. Often they appear as paired elements in the parallel chromonemata, although such an arrangement has not been observed as a constant one. No clue as to the possible function of the chromomeres is offered.

In the progression through the prophases the affinity for the stain is gradually increasing, which fact, as in the anaphases, makes it difficult to discern internal structure. There is never a question as to the duality of the elements of the chromosome. The split halves are always pronounced even though they remain twisted around each other until the equatorial-plate stage. The problem of greatest concern is the precise nature of the internal reorganization occurring within each of the threads. Certain of the threads shown in figure 47 give faint indications of containing a pair of spirals (see thread at *a*). The evidence is too slight to be valuable, although I do not doubt that internal differentiation has occurred. However, at the time of the dissolution of the nuclear membrane (fig. 49) one of the half chromosomes shows the detail indicated by figure 50. Certainly there is a distinction here between two substances of different refractive indices and different colloidal characteristics. I found it impossible to trace step by step the internal adjustment which must have been occurring in each of the parallel threads of the prophase chromosome prior to this time. Just how far back the readjustment dates and to what degree organization exists remains a mystery. The first accurate information is offered by figure 48. This projecting end of a late prophase chromosome between the stages represented by figures 47 and 49 shows most pronounced bulges in each thread. Three pairs of these bulges are visible, the upper pairs connected by delicate anastomosing processes. Within each bulge a lighter-colored area is seen, suggesting achromatic material. It is thus apparent, and this observation is verified at subsequent stages, that there are present at this time in each of the chromosome halves both the chromatic and the achromatic systems. It is difficult to determine whether the chromonemata are completely organized. Yet the bulges are highly suggestive of the spiral threads. And again their paired relationship recalls the so-called chromomeres of the telophases and the similar granules of the earlier prophases. In light of this fact, I believe these chromomeres are the first visible evidences of differentiation within the thread, a differentiation which ultimately expresses itself in a distinction between the chromatic and the achromatic substances. The former of these is to appear as the chromonemata of the subsequent generation.

### Metaphases

As the nuclear membrane disappears and the chromosomes stretch across the equatorial plate, although their halves may be considerably intertwined they show the undulating outlines suggesting their internal structure. The notched extremities and the hollow-rod appearance in end view are also indicative of the presence of both the chromatic and the achromatic systems. The pronounced median constriction is most strikingly shown in figure 53. Bead-like terminal appendages somewhat suggestive of satellites are seen in figure 51.

Untwisting continues until the halves lie side by side as parallel rods (fig. 54). Structure within these rods is shown in certain parts of this figure but is more clearly portrayed within the single limb seen in figure 55. From a review of many chromosomes at this period it appears probable that at times internal reorganization may still be proceeding, so that a continuous chromonema is not always traceable. It can with certainty be determined, however, that the halves of the chromosomes are not the solid rods usually figured for this stage. They are composed of both chromatic and achromatic materials, with the former visible in the form of a double spiral thread. It follows that, so far as the chromatic material is concerned, the chromosome is a four-parted structure, a pair of parallel halves each containing duality of elements. That the parallel halves will separate during the metaphases has been shown above. The elements within the halves are designed to separate in the metaphases of the succeeding mitosis.

### DISCUSSION

#### Structure of the Chromosomes

During the late prophases, the metaphases, and the anaphases, it is possible to distinguish within a somatic chromosome both chromatic and achromatic materials. Verification of the existence of these dissimilar substances is offered by the metaphase chromosomes of the first meiotic division both in this plant and in *Rhoeo discolor* (figs. 60-65). From such figures as these it is apparent that the oxychromatin is a matrix supporting the basichromatin, and not a core on the surface of which is found the chromatic material. The latter view was held by Bonnevie (1908, 1911) for *Ascaris* and Allium, de Horne (1911) for *Salamandra* and Allium, and Vejdvský (1912) for *Ascaris*.

Sands' aceto-carmin preparations were valuable in demonstrating the achromatic material as the substance in which the chromatin was imbedded. That the latter was peripherally disposed was verified by the living chromosome dissected from the pollen mother cells of this plant by Chambers and Sands (1923), who described an elastic cylinder, possessing a cortex which differs markedly in refractive index from the central core.

Since the oxychromatin is the continuous, bounding substance of the

chromosome, it is not unlikely, as suggested earlier, that a limiting although invisible membrane is present. The chromosome is a complex of systems, colloidal and molecular. At least two of these systems, undoubtedly colloidal, can be distinguished. They have been designated as chromatic and achromatic. The chromosome is in contact with the cytoplasm, another colloidal system. Between the latter two an interfacial membrane is present, undoubtedly not a surface-tension membrane, that is, monomolecular, but of colloidal dimensions. Its existence is most probable, as noted by Seifriz (1921) for other protoplasmic bodies. As this worker has stated, "the chemical and physical forces active in the septum separating two fluid systems are often sufficient to convert the septum into a third system differing physically and chemically from either of the two systems which it separates."

I believe a degree of verification is offered by the series of figures 56-63 showing fixation gradations of a half chromosome during the metaphases of the first maturation division from a solid rod to a pair of parallel spiral threads. Ability of the basichromatin to move freely within the limiting membrane would account for these and similar fixation appearances. In figures 62 and 63 the bounding membrane is removed to some distance from the chromonemata. This condition is caused by the pressure of smearing and is perhaps to be ascribed to the forcing out of the achromatic material, the interfacial membrane being removed from its normal position. There is in these figures the suggestion of a vesicle such as was reported by Janssens (1905) for *Batrachoseps*, Vejdovský (1912) for *Ascaris*, Lee (1920, 1924) for *Salamandra* and Paris, and Martens (1922) for Paris. I have failed to find any such vesicle around the somatic chromosomes of *Tradescantia*. However, the appearance of a vesicle would be simulated if the chromatic material, instead of being appressed to the bounding membrane, were imbedded more deeply in the matrix.

During the telophase changes the achromatin appears to form or to become a part of the karyolymph. Bonnevie and Vejdovský concluded similarly. Consideration of the sacs formed by the ends of chromosome arms (for example, fig. 31, Pl. VII) shows that the karyolymph occupies the same position previously held by the oxychromatin, the membrane of the chromosome being now more pronounced as a nuclear membrane. This membrane of the single nucleus replaces the many limiting layers of the nucleus-like chromosomes.

### The Chromonema Theory

In the foregoing pages an attempt was made to trace the chromonemata as persistent structures through the various phases of the mitosis. Schneider (1910) succeeded in this respect, but most authors who have described the chromonema saw the spiral thread of some stages lost or replaced by some other type of structure during the remainder of the nuclear division.

It is fortunate that the observation of the chromonemata by Baranetzky

antedated the ascendancy of the paraffin method, the more so because of the present revolt against conclusions based solely on the coagulation products of fixed material. Baranetzky's analysis of the chromosomes of *Tradescantia* seems to have lost much of its value with the description of the alternating discs in the same genus by Strasburger (1882) and in other genera by Balbiani (1881), Carnoy (1884), and Korschelt (1884). The association of alternating discs and spiral threads is apparently a close one. Baranetzky saw both but regarded the latter as the correct interpretation. Van Herwerden (1910) compared her preparations of the nuclear thread in the salivary glands of the *Chironomus* larva, in which she saw spirals, with those of Bolsius (1911) who saw alternating discs. The workers agreed that the thread varied in the different species used and that evidence was offered of the polymorphic structure of the chromatic body. Alverdes (1912) attempted to reconcile the divergent views by finding discs present in very young and very old larvae but double spirals arising from and passing over into the discs. Vejdovský (1912) saw the chromonema originating during the prophase within the then extending thread persisting from the preceding mitosis. But the newly formed spiral broke up into a series of alternating discs prior to the longitudinal splitting of the old chromonema.

The notion that the disrupted, discontinuous chromonema may be the result of certain types of fixation distortion is emphasized by a comparison of the reaction to the aceto-carmin mixture as figured by Sands (1923) and the definite spiral portrayed in this paper. Despite his description of the chromatic material as chromomeres variable in size, shape, and number, Sands figured and mentioned indications of the spiral structure (*cf.* page 62 of the present paper, also Sands' figs. 5 and 10, chromosome *a*). It seems a safe conclusion that the uninterrupted spiral of Baranetzky's report and of this paper, rather than any fragmentation, should be recognized as the normal condition. That any series of artifacts could persistently result in a formation so uninterrupted as the chromonemata appears to be beyond the limits of probability. Wilson (1912) likewise discredited vacuolation, corrosion products, or fixation artifacts as an explanation of the spirals found in the spermatogonial prophase of Orthopteran material.

Sands unfortunately made no attempt to figure the chromosome through the cycle of mitosis. Martens (1922), however, based his interpretation of mitosis in *Paris* on a discontinuous chromonema. The splitting of the prophase spirem did not occur along but really across the chromonema, in consequence of a bilateral repartitioning of its material. Connections between the half chromosomes were persistent from the repartitioning and not pseudopodium-like outgrowths. Divergent as this interpretation may seem from that herein applied to *Tradescantia*, and despite the fact that different genera are considered, there seems some basis for comparison. It must be remembered that the double spiral threads have been figured in the telophases of *Paris* by Lee (1924), although he believed these to be



intertwined halves of the transversely split chromosome. There are certain objections to the application of Martens' interpretation to *Tradescantia*. (a) The chromonemata are not discontinuous, although under certain conditions of artifact they so appear (fig. 5, Pl. VI). (b) The splitting of the chromosomes is not a prophase occurrence. (c) Processes from the chromonemata not only pass between the threads but also extend out into the karyolymph. (d) The prophase chromosomes are tightly interlaced in many cases, even when the halves are most delicate threads (fig. 41, Pl. VII). When they come to lie as extended parallel rods the prophases are well advanced (fig. 44). The threads do not split and then intertwine; they are twisted around each other from the time of their origin.

The literature holds no dearth of description of prophase spirals (Janssens, 1901; Wilson, 1912; Wenrich, 1916; Newton, 1924). It is during the later stages of this period that I have seen the first complete evidence of the endogenous formation of the new chromonemata. They originate within the chromomere-like structures, which accordingly must be centers of greatest physical and chemical readjustments. Whether the occasional paired chromomeres of the telophases (fig. 16, Pl. VI) are perpetuated during interkinesis is a problem still within the realm of the conjectural. It is evident, however, that a remarkable preparation for ensuing mitoses is made far in advance. If the telophase chromomeres are concerned in chromonema-formation, they are related to the spirals becoming visible during the following prophases which will not separate until the metaphases of the succeeding nuclear division. The disruption of the endogenous chromonema of the prophase threads of *Ascaris* as Vejdoský figured it was a complication associated with the presence of one thread instead of a pair of threads. This complication did much to shatter the force of Vejdoský's argument.

Again, there is no dearth of descriptions of the chromonema during the telophases (Bonnievie, 1908, 1911; Schneider, 1910; Lee, 1920, 1924). Lee's interpretation of intertwined halves of V-shaped chromosomes can not possibly be applied to *Tradescantia*, since the two threads are visible in each of the limbs of the anaphase chromosome. Lee discounted as "indubitably erroneous" such suggestion of a longitudinal anaphase split, since he saw in *Salamandra* and *Paris* a spiral which was a mere rib or surface expression of the chromosome. For the twisting of the halves a polar clumping occurred at the culmination of the anaphase, a condition normally non-existent in *Tradescantia*.

It is the metaphases and the anaphases which have offered the least confirmation of the chromonema hypothesis. Certainly the spirals are present at this time in *Tradescantia*, although the difficulty of fixation is greater than during the other phases. The aspect of the quadripartite chromosome of these stages is perhaps but the casual manifestation of the disrupted chromonema. In certain of the preparations of the metaphase

chromosomes of the first meiotic division in *Tradescantia*, and more especially in *Rhoeo*, the simulation of a four-parted structure is striking.

### Duality of the Chromonemata

There is a striking contrast between the figures of the telophase chromosomes shown in this paper and the irregularly vacuolated structures pictured by Sharp (1920). Some workers who have seen vacuoles have regarded them as median and indicative of a longitudinal split with an ensuing duality of elements. Sharp has suggested that the vacuolation is not median but decidedly irregular. Thus the chromosome of the telophases can be no more called double than triple or quadruple.

On a basis of my preparations, were this irregular telophase vacuolation assumed to occur, I should be forced to account for the disruption and alteration of the two filaments of the anaphase chromosome. That these filaments are actual structures and not the images of the walls of alveoles, as the proponents of the alveolation hypothesis would have us believe, has been proved by certain details of description above.

Too much valuable evidence has been advanced by cytologists working on both plant and animal material demonstrating a longitudinal split during the anaphases or earlier to allow an entire negation of the idea. A most striking additional proof is furnished by the presence of a more or less completely split satellite appendaged from the arm of a late anaphase chromosome of *Gasteria* (Taylor, 1925). Nor should the fact be overlooked that Bonnevie, one of the advocates of the single-chromonema hypothesis, has figured duality during the anaphases, telophases, and prophases (see her figs. 72, 42, 64). To her this was the unusual and not the normal condition. Yet, is it not more likely that the single thread is a fixation product caused by the lateral approximation of parallel filaments so that the split can no longer be observed?

Disregarding, however, any evidence of anaphase duality, there is for consideration the premise of Sharp that the telophase alveolation is decidedly irregular and that no true duality occurs. As I have traced the changes during the telophase, there are introduced the factors of transformation of the oxychromatin and the translocation of the basichromatin coincident with the thinning of the chromonemata. During the gradual attendant changes it is not impossible to picture the chromosome as an alveolated body if the density of color be the only guide. However, the most diagrammatic intertwined threads have been seen. I am willing to agree that the occasional appearance of a double spiral might readily be explained away on the basis of the aspect of alveoles. But can the appearance of those spirals in a number of chromosomes in a single nucleus be so diagnosed? Another factor which must not be overlooked is the tendency of the chromonemata to send out anastomosing processes. Since these bridge the gaps between the threads, they complicate the analysis of the structural units, cause alveolation appearances, and even obscure the split.

That alveolation is a fixation artifact in this plant is a conclusion emphasized by a consideration of the metaphase chromosomes of the first maturation division shown in figures 56-63, Pl. VIII. Gradation from the evenly staining rod to the double spiral bands is made through a series of magnificently vacuolated structures. Figure 60 as a connecting link clearly portrays how anastomosing between the turns of the coil soon obscures the more precise, correct detail seen in figures 61-63.

The persistence of the dual filaments is rendered questionable by the apparently unsplit threads of some early prophase nuclei (fig. 34, Pl. VII). Were it not possible to find abundant evidence of double structures in these nuclei, the problem would be seriously complicated. However, since only some of the chromosomes, and particularly those stretched across the diameter of the nucleus, have the split obscured, it seems likely that in the stretching and lengthening an approximation of parallel filaments has taken place. This is the stage at which Sharp believed splitting takes place through vacuolation. Lee (1924) abandoned his earlier idea of prophase vacuolation in *Paris* (1912) in favor of the interpretation of closely intertwined spiral threads. The tendency of parallel threads to approximate to the point of completely hiding the split is well recognized. For example, Sharp's figure 24 of a late prophase in *Tradescantia* reveals no split, although there is no question of duality at that time in my preparations. The individuality of parallel threads may be concealed to a great extent by the anastomosing processes between them. Proponents of the vacuolation theory regard these processes as residual from the unvacuolated portion of the thread. But it must not be forgotten that the processes connect not only halves but also adjacent chromosomes. Similar projections into the karyolymph have been figured by Bonnevie (1911, fig. 8, Pl. X) and de Horne (1911, fig. 15, Pl. XXXVI).

When the chromonemata are traced through the entire mitosis, it is apparent that they are but parallel halves which differ from the ordinary rods in assuming a coiled form. This phenomenon is perhaps associated with conservation of space, since the coil is a more compact unit than the extended thread. Coincident with the coil is the introduction of chromonema-formation endogenously, a more complicated process than the longitudinal splitting of the rod-shaped structure.

As to any linear arrangement of genes in this plant, the rod-like chromosome can no longer be considered as a unit. Linear arrangement must follow the course of the chromonemata, since they alone maintain the genetic continuity of the chromosome. If the chromonemata are disregarded and the genes are assumed to be located along the greater axis of the chromosome, there would be introduced the possibility of the three-dimensional arrangement suggested earlier by Castle (1919) but more recently discounted by the same author (1921).

## SUMMARY

1. The anaphase chromosome is divisible into two morphologically dissimilar substances, one of which, the achromatic, serves as a matrix bearing the other, the chromatic substance. The chromatic substance exists in the form of a pair of unbroken, intertwined spiral threads or chromonemata.

2. At telophase the achromatic substance becomes continuous with the karyolymph, the chromonemata maintaining the genetic continuity of the chromosome.

3. Individuality of the chromosomes and of the chromonemata is apparently not lost during interkinesis.

4. In the early prophase the chromonemata are sometimes seen as distinct intertwined threads, again as a single thread spirally coiled. The latter appearance is probably due to the lateral approximation of parallel filaments sufficient to obscure the split.

5. As the prophase advance, chromomere-like swellings appear in the thread. Each of these can later be demonstrated to be a center of activity concerned in the formation of the new chromonemata.

6. At the equatorial-plate stage the chromosome consists of a pair of parallel threads, each of which shows achromatic and chromatic materials, the latter visible as a pair of spiral threads.

7. Vacuolation, as shown by the metaphase chromosomes of the first meiotic division, is in this plant an appearance caused by artifact, and may be partly due to anastomoses between the chromonemata.

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### DESCRIPTION OF PLATES

All observations were made using a Spencer no. 14JP microscope equipped with N.A. 1.40 condenser. All figures were made at the level of the table with the aid of a camera lucida at a magnification of 3,100 diameters, using a Zeiss 2 mm. apochromatic objective and a Zeiss 20x compensating ocular. In reproduction, all except figure 35 are shown at a magnification of approximately 2,050 diameters. Figure 35 is shown at 3,100 diameters.

Figures 1-26, 28-55: Somatic mitoses, *Tradescantia pilosa*.

Figure 27: Premeiotic division, *T. pilosa*.

Figures 56-63: First meiotic division metaphase, *T. pilosa*.

Figures 64, 65: First meiotic metaphase, *Rhoeo discolor*.

### PLATE VI

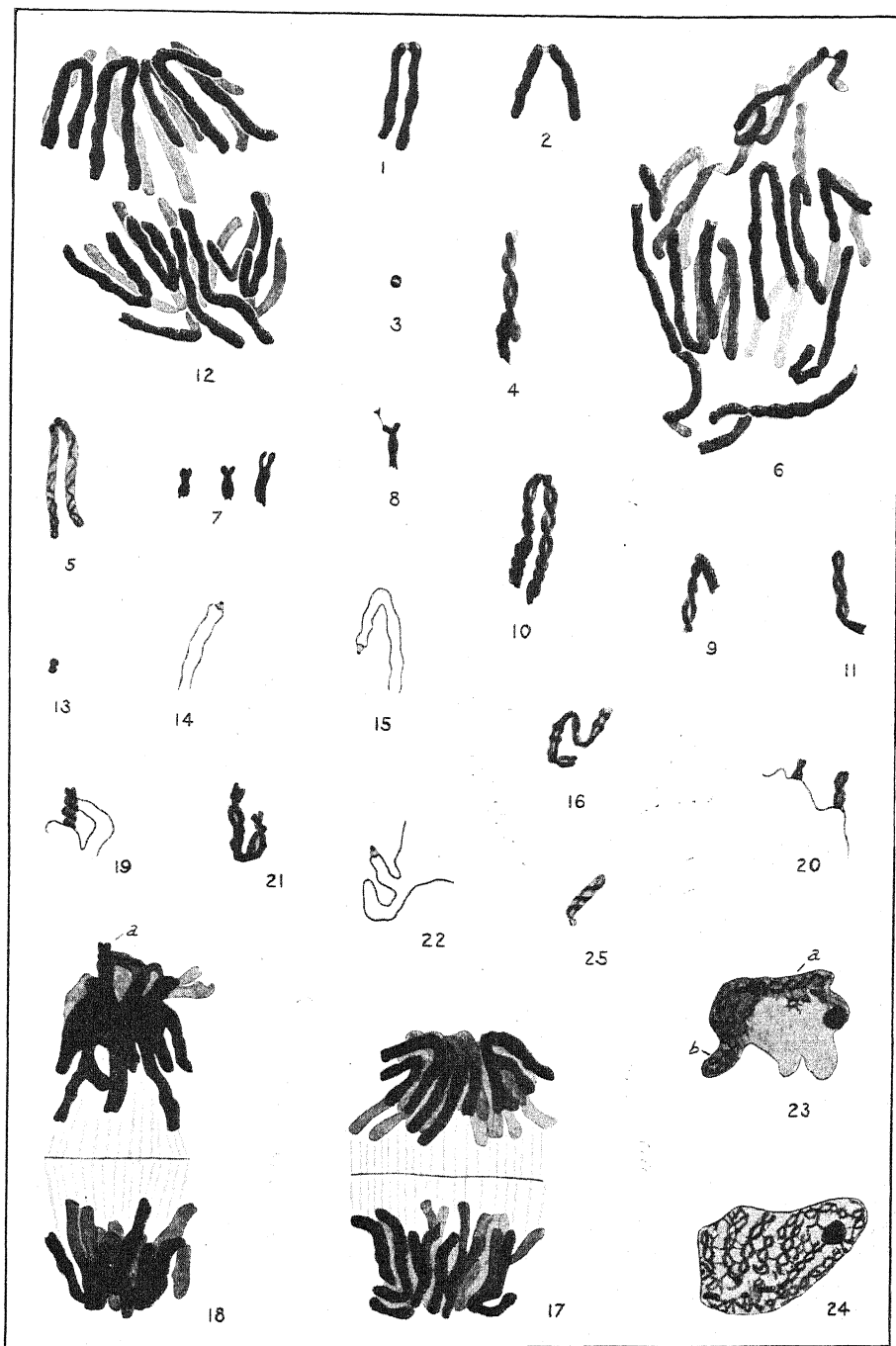
- FIG. 1. Anaphase: V-shaped chromosome showing narrow median constriction.
- FIG. 2. Ditto: Wider constriction.
- FIG. 3. Ditto: End view, one arm of the chromosome showing a duality of chromatic elements.
- FIG. 4. Ditto: Intertwined chromatic threads or chromonemata.
- FIG. 5. Ditto: Discontinuous chromonemata, a fixation distortion.
- FIG. 6. Ditto: Chromosomes moving to the poles; note the single pair of terminal satellites.
- FIG. 7. Ditto: Terminal indentations suggesting a duality of chromatic elements.
- FIG. 8. Ditto: Torn chromosome, verifying the same suggestion.
- FIG. 9. Ditto: Chromosome at the stage shown in figure 12, illustrating chromatic and achromatic substances.
- FIG. 10. Ditto: Same at a slightly later stage.
- FIG. 11. Ditto: Same.
- FIG. 12. Late anaphase: Undulating outlines of chromosomes indicative of spiral threads.
- FIG. 13. End view of a chromosome arm at the stage shown in figure 12.
- FIG. 14. Ditto: Terminal satellite imbedded in an achromatic matrix.
- FIG. 15. Ditto: Same.
- FIG. 16. Ditto: Portion of a chromosome in which untwisting has occurred. Note the paired chromomere-like swellings.
- FIG. 17. Ditto: Grouping of chromosomes at poles.
- FIG. 18. Ditto: Apparent fusion of members of the upper group in consequence of density of color and mass of material.
- FIG. 19. Detail of chromosome *a*, figure 18.
- FIG. 20. Projecting ends of chromosome arms at the same stage.
- FIG. 21. Isolated chromosome of the same stage; chromatic and achromatic regions distinguishable.
- FIG. 22. Telophase: Satellite on the projecting arm of a chromosome.
- FIG. 23. Ditto: Chromonemata persisting, achromatic material becoming continuous with the karyolymph.
- FIG. 24. Later stage: Contrast of chromonemata and karyolymph more decided. Anastomoses pronounced.
- FIG. 25. Diminution in diameter of chromonemata.

## PLATE VII

- FIG. 26. Telophase: Nucleolus attached to chromosome.  
FIG. 27. Ditto: Premeiotic nucleus.  
FIG. 28. Ditto: Pronounced anastomosing between chromosomes.  
FIG. 29. Ditto: Limits of chromosomes becoming less definite because of anastomosing processes.  
FIG. 30. Later stage. At *a*, zigzag aspect of chromonemata.  
FIG. 31. Ditto: Zigzag threads in a sac formed by a chromosome arm.  
FIG. 32. Interphase: Reticulum simulated by pronounced anastomosing; at *a*, chromonemata visible.  
FIG. 33. Prophase: Anastomoses breaking down; duality of threads visible in several places.  
FIG. 34. Ditto: Pronounced chromonemata. Chromosome *a* shows double threads; similar appearances in other places.  
FIG. 35. Ditto: Showing how a more open spiral can grade over to very close threads.  
FIG. 36. Ditto: Nucleolus removed from the nucleus by a knife and carrying with it an attached chromatic thread.  
FIG. 37. Ditto: Section of a nucleus which did not form a reticulum, indicating pronounced anastomosing between chromonemata.  
FIG. 38. Later stage: Anastomoses withdrawn; compound nucleoli appear.  
FIG. 39. End view of chromosomes at the stage shown in figure 38.  
FIG. 40. Ditto: Compound nucleolus and attached chromosomes.  
FIG. 41. Later stage: Loosening of coils. Pseudopodium-like processes of the surface of chromonemata pronounced.  
FIG. 42. Nucleolus from the nucleus of figure 41, showing attached chromosomes.  
FIG. 43. End view of chromosomes from the same nucleus.  
FIG. 44. Later stage: Uncoiling and thickening of thread.

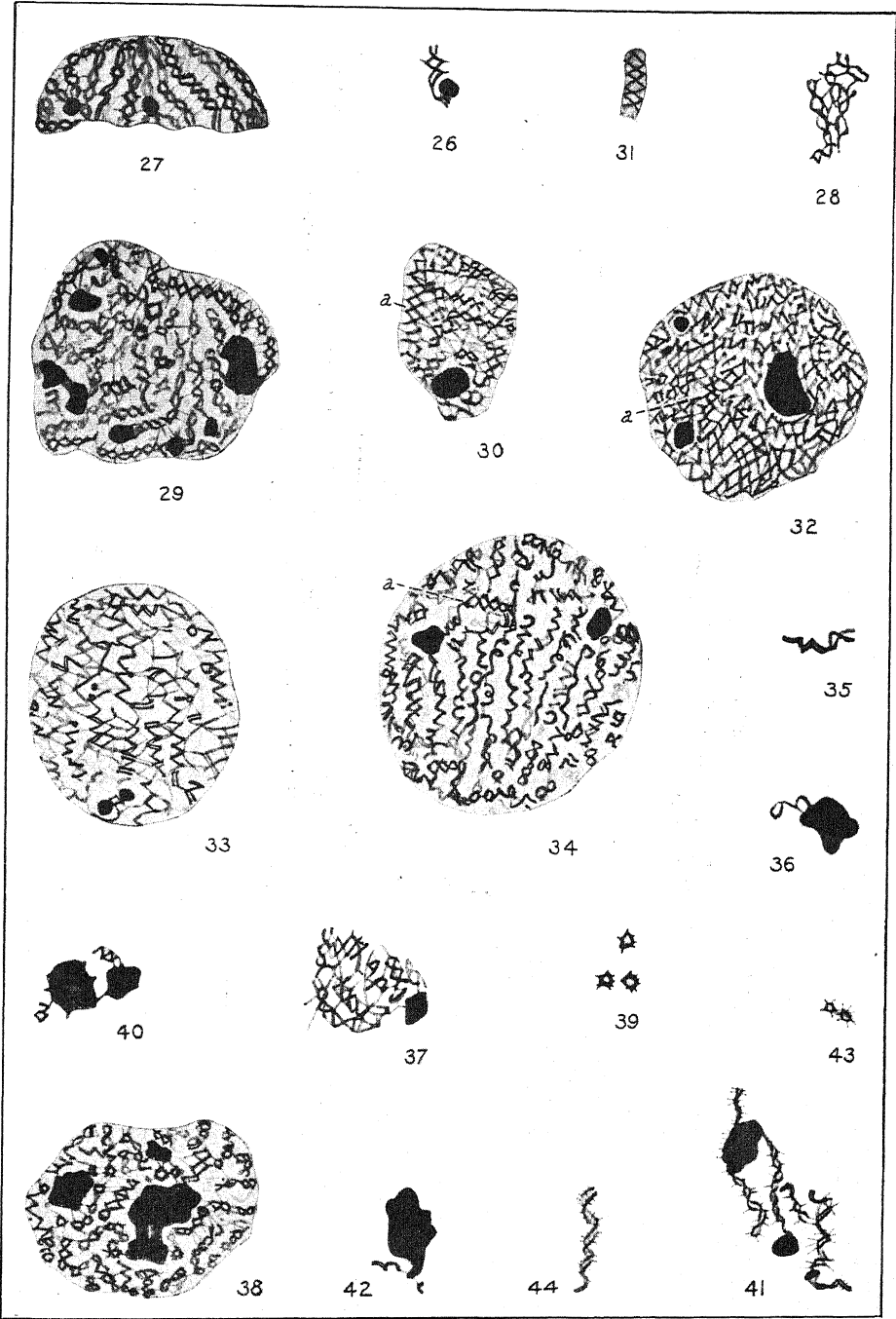
## PLATE VIII

- FIG. 45. Late prophase: Uncoiling has occurred in some chromosomes but not in others. Anastomoses are pronounced across spaces between halves as well as between chromosomes.  
FIG. 46. Ditto: Chromomere-like structures seen.  
FIG. 47. Ditto: Shortening and thickening proceeding. At *a*, traces of the formation of the internal spiral.  
FIG. 48. Ditto: Chromomere-like structures of earlier stages now concerned in the formation of new spirals. Chromatic and achromatic materials visible.  
FIG. 49. Ditto: Chromosomes at the time of the dissolution of the nuclear membrane.  
FIG. 50. Half chromosome at a stage comparable to that of figure 49.  
FIG. 51. Equatorial-plate stage: Suggestion of satellites.  
FIG. 52. Ditto: Halves tightly intertwined.  
FIG. 53. Ditto: Pronounced median constriction.  
FIG. 54. Ditto: Untwisted chromosomes. In places the halves show internal spiral chromatic threads.  
FIG. 55. Arm of chromosome at the stage shown in figure 54, illustrating the chromonemata.  
FIGS. 56-63. Diads of the first meiotic division. This series shows fixation gradations from the solid rod through vacuolated structures to the single, and eventually the double, spiral threads.  
FIG. 64. Diad of a metaphase of the first meiotic division in *Rhoeo discolor*. The achromatic matrix bears chromonemata.  
FIG. 65. Tetrad of a metaphase of the first meiotic division in the same plant.

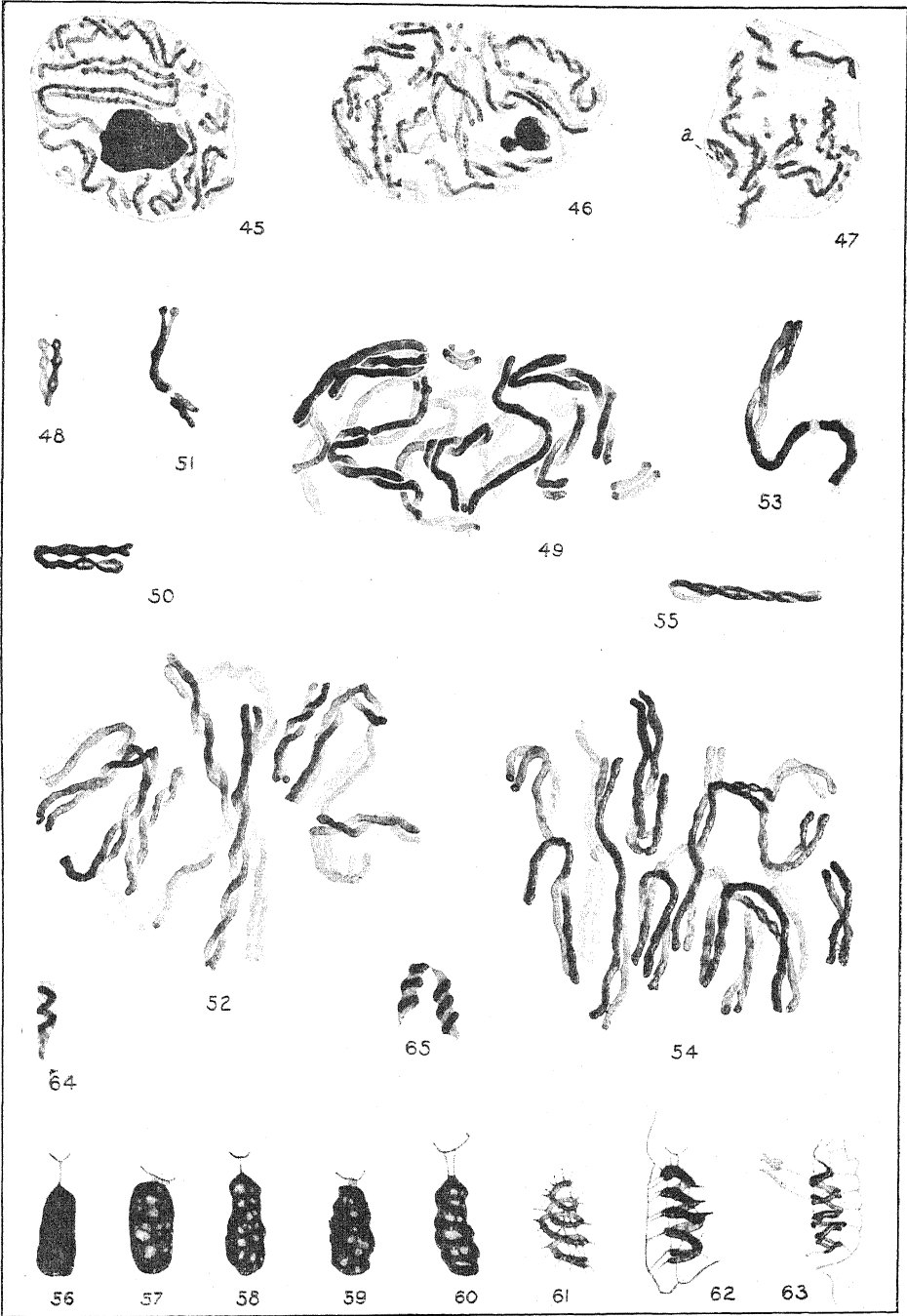












KAUFMANN: CHROMOSOME STRUCTURE



# THE EFFECTS OF SULFUR DIOXID UPON PLANTS:

## METHODS OF STUDY

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The contamination of the atmosphere with sulfur dioxide has long been thought a detriment to the growth of plants as well as to the health of man. The supposedly harmful effects of the gas have been responsible for frequent litigation between farmers and foresters on the one hand and smelters and manufacturers on the other. The problem of the determination of such effects is of particular interest wherever smoke accompanied by sulfur dioxide is emitted from furnaces, coke ovens, or other combustion systems.

The original purpose of the writer was to study the effects of sulfur dioxide upon vegetation. It was soon found that known methods of experimentation were subject to certain errors, and that many problems in the method of conducting an experiment must first be solved. This work is therefore a study of method; it will probably be continued by applying the method developed to a study of the effects upon plants of air contaminated with traces of sulfur dioxide.

### HISTORICAL REVIEW

Methods for investigation of the effects upon vegetation of air contaminated with sulfur dioxide have been varied. Wislicenus, in 1897, determined sulfur dioxide by suspending in the air cotton saturated with barium carbonate to absorb sulfurous and sulfuric acids. After five to seven months the cotton was collected and the absorbed substances were determined. Weiler (1906) analyzed air by drawing 1,000 to 2,000 liters through potassium carbonate over a period of six hours, and the sulfur dioxide absorbed was weighed, after oxidation and precipitation, as barium sulfate; later Weiler substituted alkaline bromine water for potassium carbonate. Lunge (1899) used sodium hydroxide as an absorption fluid. After the absorption, one half of the solution was treated with barium chloride to precipitate sulfur trioxide, and after addition of bromine water the other half was similarly treated to precipitate both sulfur dioxide and trioxide.

Among others who used gravimetric methods for air analysis were Gerlach (1909), who used potassium carbonate with bromine as the absorption fluid, Herbig (1909), who used a sodium hypobromite solution, and Ebaugh (1907), who drew air through an iodine solution. Gallagher (1909) used a hydrogen peroxide solution. Ascher and others (1908) drew filtered

air through potassium iodid, and later substituted hypo-iodous acid to which iodine crystals had been added for potassium iodid.

Ost (1899) drew 34.5 liters of smoke containing sulfur dioxid through a 0.1 *N* iodine solution in four and one half hours; after absorption, one half of the solution was titrated with 0.1 *N* thiosulfate and the remainder was treated with barium chlorid. Lehmann (1893) used 0.025 *N* iodine solution and 0.025 sodium thiosulfate solutions for large volumes of samples. Frazer (1907) used barrels fitted with cloth diaphragms, which were saturated with alkali carbonate; by the increase in the weight of the cloth the quantity of sulfur trioxid absorbed was determined. Reich (1917) determined the percentage of sulfur dioxid in air by finding the size of the sample needed to decolorize one cubic centimeter of iodine solution of known concentration; the Reich apparatus used a rubber stopper near the solution. Seidell and Meserve (1914) analyzed the air of railway tunnels by allowing a sample of air to rush into a partially evacuated flask containing a dilute starch solution, to absorb the mixture; the starch solution was then titrated with 0.001 *N* iodine solution. Holmes, Franklin, and Gould (1915) made air analyses by allowing a sample of air containing sulfur dioxid to rush into a partially evacuated flask containing a dilute starch solution; after absorption, the solution was titrated back to the color of a "blank" by means of a 0.001 *N* iodine solution. Rubber stoppers were used on these flasks.

Among those who conducted controlled experiments for a study of the problem was Wislicenus (1898) who exposed plants to sulfur dioxid obtained by burning known amounts of carbon bisulfid mixed with alcohol to a definite volume. The concentration of sulfur dioxid in the resulting air was calculated by the rate of burning of the carbon bisulfid-alcohol mixture. Later (1914) he liberated a stream of sulfur dioxid from a cylinder into the gas chamber; he used a 0.01 *N* solution of chromic acid as an absorption fluid to determine the concentration of the gas. Haywood (1905) conducted experiments according to the early methods used by Wislicenus. Kastle and McHargue (1907) determined the sulfur dioxid obtained in an air mixture by burning 0.1 gram portions of sulfur, using 0.1 *N* iodine and sodium thiosulfate solutions; they used comparatively large volumes of samples. Stoklasa (1923) exposed plants to sulfur dioxid obtained by burning a mixture of carbon bisulfid and alcohol. No method of analysis of the air for its sulfur dioxid content is described, although it is stated that the mixture analyzed had a lower concentration of gas than that calculated from the weight of the carbon bisulfid burned.<sup>1</sup>

#### GENERAL STATEMENT AS TO EQUIPMENT

The equipment used in the early experiments herein described differed from that employed in the later ones as various improvements were adopted from time to time. The final apparatus only is here discussed. The gas

<sup>1</sup> Since the above was written, a letter has been received from Dr. Stoklasa stating that the gravimetric method of determining the concentration of sulfur dioxid was used.

chamber used throughout the investigations was built in a greenhouse. It was enclosed by double sashes, and all joints, after being tightly sealed with putty and paint, were covered with shellac, a treatment that was necessarily repeated at frequent intervals. The chamber was 2.1 meters long and 0.67 meter wide; the top sloped toward the south, giving a height at the front of 1.3 meters and at the back of 0.95 meter, with a capacity of 1,610 liters. The chamber had two tightly fitting doors, one opening into the greenhouse and the other out of doors; the door into the greenhouse had a circular opening about 10 inches in diameter, devised to enable the investigator to reach into the case without loss of sulfur dioxide. The opening was carefully sealed with a piece of sheet rubber with the center cut sufficiently to fit tightly about the wrist when a hand was put through the opening; when not in use the hole in the rubber was kept closed by means of a Hoffman clamp. The opening was further fitted with a wooden disc, gasketed with a felt strip which insured against leakage. The chamber was equipped with a wet-and-dry-bulb psychrometer, and with an electric fan to mix the gas; the blades of the fan were coated with shellac.

The investigations herein discussed were carried on in the botanical laboratories of the University of Pennsylvania and covered the period of time from October, 1922, to July, 1924.

The writer wishes to express her sincere appreciation to Dr. R. H. True for the stimulus of his untiring interest and generous assistance throughout the work. Acknowledgment and thanks are expressed to Dr. W. D. Langley for his active interest and valuable suggestions; to Dr. W. R. Taylor for his generous aid and advice given in the preparation of the plates and the text figures; and to Dr. C. H. Arndt for valuable criticisms of methods and apparatus.

#### METHODS OF GENERATING SULFUR DIOXID

*From Sulfur.* In the early investigations, arsenic-free sulfur, calculated to give a desired concentration of sulfur dioxide, was weighed in a small crucible and burned within the gas chamber. It was found difficult to burn the small quantities which were to be used (e.g., 0.0229 gram calculated to give a concentration of 0.001%, or 10 parts of sulfur dioxide in 1,000,000 parts of mixture). For complete combustion of the sulfur it was necessary to use an alcohol lamp to melt the sulfur before it was burned. Thus it was possible for acetaldehyde to be produced which, with the sulfur dioxide, seemed likely to produce a different effect upon plants from that produced by sulfur dioxide alone. Consequently an attempt was made to bring an electrically heated coil of platinum wire in direct contact with the sulfur; it was found difficult to control the resistance in the circuit, and as a result the wire fused. Further, some sulfur trioxide, in addition to sulfur dioxide, was produced by this method and it was therefore abandoned.



The sulfur was next burned by being placed upon the bottom of an inverted Pyrex beaker which was placed into a circular opening in the bottom of the chamber, over a heating unit from a commercial electric heater. The coil and beaker were insulated and surrounded with a 6-centimeter packing of asbestos cement. The inverted beaker served the three-fold purpose of (a) protecting the wire coil from the action of the sulfur dioxide, (b) of insulating the coil, thus avoiding a rise in temperature, and (c) of keeping the case tightly sealed. When an experiment was to be conducted, the current was turned on and the weighed sulfur, on thin platinum foil, was put on the inverted beaker. The gradual rise in temperature resulted in the production of some sublimed sulfur. Further sources of error in the method of burning sulfur were found to be: adsorption on the glass walls, absorption by the soil and other agencies, and a relatively rapid oxidation, so that the concentration of sulfur dioxide obtained was less than that expected from the amount of sulfur burned. The method of burning sulfur to obtain sulfur dioxide was therefore abandoned, and other means of generating the gas were employed.

*From Sodium Bisulfite.* A 10% solution of sulfuric acid was allowed to drop upon a saturated aqueous solution of sodium bisulfite, and the sulfur dioxide evolved was washed by being passed through another saturated aqueous solution of sodium bisulfite and sulfur dioxide. It was necessary to adapt apparatus for testing the purity of the sulfur dioxide, for collecting and measuring it, and for conducting it into the chamber where it was to be diluted with air before plants were exposed to it. The manipulation of the gas presented a number of difficulties caused by changes in volume due to changes in temperature and in pressure and by adsorption of the gas, as mentioned under the heading "Procedure for an Experiment." The apparatus, of which Plate IX is a photograph, is discussed under three headings, namely, the generating apparatus, the gas chamber, and the apparatus for withdrawing and analyzing the sample.

The generating apparatus (*a* to *p*, Pl. IX) consisted of a one-liter Erlenmeyer flask, *a*, placed in a water bath, and containing a suspension of sodium bisulfite in water. It was fitted with a three-hole rubber stopper through which were inserted a separatory funnel, a tube with stopcock connected to the equilibrium flasks, *g* and *g'*, and a tube with stopcock to conduct the gas into the 500-cc. washing bottle, *h*, containing distilled water saturated with sodium bisulfite and sulfur dioxide. The inlet tube of this bottle was terminated by an aërating bulb. The outlet of the washing bottle led to a three-way stopcock, *i*, which was connected by one arm to a second three-way stopcock (seen back of *i*, Pl. IX), and by the other arm to the collecting cylinder, *l*. From the second three-way stopcock the gas could be sent either out of doors through the Y-tube, *k*, or to the testing cylinder, *a*.

The second arm of the first stopcock, *i*, which leads to the collecting cylinder, *l*, was connected to an inlet tube reaching nearly to the bottom of

the calibrated collecting cylinder filled with 800 cc. of water saturated with sulfur dioxide and sodium bisulfite. The outlet, *n*, from this cylinder led to an opening in the gas chamber, and a tube from an opening at the bottom of the cylinder was connected, by means of a shellacked rubber hose, to the bottom of a leveling flask, *m*, which was operated by a rope-and-pulley system. The rubber stopper in the top of the leveling flask had two holes, one fitted with an overflow tube leading from the bottom of the flask, so that only the surface of the column in the overflow tube came in contact with the outside air. The other hole was fitted with two U-tubes, in series, of which the first contained a mercury trap and the second sodium hydroxide to absorb any sulfur dioxide which escaped through the mercury of the first.

A safety device to control changes in pressure and volume was that of two 2-liter Erlenmeyer flasks, *g* and *g'*, connected in series between an outlet of the generator and one arm of the Y-tube, *k*, leading to the opening out of doors. With fluctuations of sulfur dioxide pressure in the generator, water saturated with both sulfur dioxide and sodium bisulfite moved from the first equilibrium flask to the second, and *vice versa*. In cases of excessive pressure, the tube from the second equilibrium flask conducted the excess gas out of doors. This device prevented the sucking back of the solutions in *h* and *l* into the generating flask, and also the sucking of air into the system when pressure or volume within decreased.

#### APPARATUS FOR GAS ANALYSIS

*Gas Chamber.* The chamber as described above was used throughout this work. During warm weather the top and outer sides were covered with white lead to diffuse the direct rays of the sun. The inner surface was later coated with paraffin. The reason for these steps will be found under the heading "Behavior of Sulfur Dioxide in Contact with Glass."

*Absorption Tubes.* Three cylindrical absorption tubes connected in series (see *x* in Pl. IX, also text fig. 4) were joined by a stopcock, *v*, to the outlet, *u*, from the chamber. The tubes were fitted with ground-glass stoppers having three openings: an inlet tube terminated with an aerating bulb leading under the surface of the iodine solution, an outlet leading to an aspirator, and an opening, *a*, fitted with a ground-glass stopper, by means of which the excess iodine could be titrated *in situ*. The tubes were 45 cm. long, and 3.4 cm. in outer diameter.

*Aspirating Apparatus.* The absorption tubes, *x*, were connected to a 12-liter aspirating bottle, *B*, to which was also connected, by means of a rubber hose at the bottom, a 6-liter graduated measuring flask, *F*, with long neck and bulb, for the purpose of leveling, or of obtaining atmospheric pressure in both the aspirator and the measuring flask. A wooden crate, *G*, attached to rope and pulleys, enabled the leveling bottle to be raised or lowered. A glass tube, *y*, connected in parallel with the absorption tubes

between the stopcock, *v*, and the T-tube leading to the 12-liter aspirator, *B*, provided means for washing air from the system before drawing the sample; for the same reason a second opening, at the bottom of the aspirator, *B*, served to draw water through the tube *E*, not connected to the measuring-flask.

*Solutions.* The solutions used in the analyses were a 0.001 *N* iodine solution containing two hundred grams of potassium iodid per liter of solution; a 0.001 *N* solution of sodium thiosulfate, prepared with freshly boiled distilled water. Various concentrations of the above-mentioned solutions ranging from 0.02 *N* to 0.001 *N* were tried, and the last-named proved to be the most satisfactory. It was carefully standardized to avoid error that is likely to result by diluting tenth- or hundredth-normal solutions. A starch solution containing one gram of soluble starch per liter of water, made fresh every day, was used as an indicator for the titration.

#### PROCEDURE FOR AN EXPERIMENT

*Generating Sulfur Dioxid.* Dilute sulfuric acid was dropped from the separatory funnel upon the saturated solution of sodium bisulfite which was warmed by means of a water bath. Occasional shaking of the generating flask was necessary to facilitate the production of gas at a uniform rate. The chemical reaction which occurred was:



The sulfur dioxid was washed in the washing bottle, *h*; it was sent out of doors *via i, j, k* until it was thought to be free of air, when the stopcock, *i*, was turned so as to send the sulfur dioxid into a cylinder, *a*, containing a sodium hydroxid solution. A tube filled with sodium hydroxid from the cylinder was inverted over the small tip of the tube, *b*; if the bubbles of sulfur dioxid coming into the inverted tube were completely absorbed, *i.e.*, if sodium hydroxid was not forced out of the inverted tube, the sulfur dioxid was known to be free from air. The stopcocks were then turned so as to send the gas into the collecting cylinder, *l*, containing water saturated with sulfur dioxid and sodium bisulfite. The sulfur dioxid entering the cylinder forced the level of the liquid down, sending it through the connecting hose at the bottom into the leveling bottle, *m*. In practice, the gas was collected under a pressure of several centimeters of water. When the volume was to be read, the gas was brought to atmospheric pressure by lowering the leveling bottle and the desired quantity of gas was sent into the chamber, *q*; with the stopcock, *i*, closed and the one between *l* and the chamber open, by means of the rope-and-pulley system, *p*, the leveling bottle was slightly raised, thus forcing the gas through the tube, *n*, into the chamber.

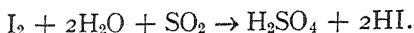
Thermometer and barometer readings were taken to furnish data necessary for making corrections in the volume of the gas. It was also

found necessary to make a correction for absorption of sulfur dioxide in the collecting cylinder during the short period that the gas was being forced from *l* into the chamber. This correction was necessitated since changes in temperature made it practically impossible to maintain equilibrium between gaseous and dissolved sulfur dioxide in the collecting cylinder; in the lower temperature of the morning the water dissolved sulfur dioxide, and evolved it as the temperature rose. For intervals of time during which the temperature varied but little, the sulfur dioxide was absorbed at a constant rate. By observing the rate of absorption for ten minutes immediately before sending the gas into the chamber, then observing the length of time used for moving the gas, the correction in the volume of gas used was easily made. In order to mix the gas, the electric fan within the chamber, near the inlet for the sulfur dioxide, was started shortly before the gas entered the chamber.

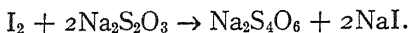
#### ANALYSIS OF THE MIXTURE IN THE GAS CHAMBER

At 15- or 20-minute intervals analyses of the concentration of the mixture in the chamber were made. A sample of the mixture was drawn from the chamber through the tube, *u*, in less than four minutes, as water was drawn from the aspirator, *B*, into the collecting flask, *F*, fully a meter below the level of *B*. Before drawing the sample through the solution it was necessary to remove air from the system. The stopcocks *v* and *z* were turned so as to draw air or mixture through the tube *y*, while water was allowed to flow from the aspirator through the tube *E*. The air having been removed, the flow of water through *E* was stopped and the stopcocks were turned to open the inlet to the series of absorption tubes, *x*; the sample was then drawn through the iodine and starch solutions in the series of tubes while water was being drawn from the aspirator into the measuring flask. When approximately six liters of water had been drawn, the measuring flask was raised by means of the rope-and-pulley system, *H*, and the water in the flask was leveled with that in the aspirator. The quantity of water drawn represented the volume of the sample minus the sulfur dioxide removed. Corrections to standard conditions were made, and the result, added to the volume of sulfur dioxide removed (a negligible volume in these calculations) represented, quantitatively, the sample of the mixture drawn from the chamber. The first absorption tube (text fig. 4) through which the sample was drawn contained an accurately measured quantity of 0.001 *N* iodine solution, which quantity varied from 20 to 80 cc. according to the concentration of gas used; the second tube, of smaller diameter (18 mm.), contained 15 cc. of starch solution so as to enable the detection of any volatilization of iodine; such volatilization did not occur when the iodine solution contained the quantity of potassium iodid specified under "Solutions." When the concentration of sulfur dioxide was estimated to be sufficiently high to reduce a larger quantity of iodine than that contained

in the first tube, a second iodine tube was put behind the first. As the sample was drawn through the solution, the sulfur dioxide present reduced the iodine thus:



The excess iodine was titrated with 0.001 *N* sodium thiosulfate until the end-point was near, when the contents of the starch tube was added to the iodine and the titration with sodium thiosulfate was completed. The following reaction occurred:



From the quantity of iodine reduced the sulfur dioxide content of the sample, hence of the chamber, was found. All calculations for these results were made as shown below:

#### Example: July 14

Water drawn, 5,990 cc.

Humidity in chamber, 38.3% ( $\therefore$  61.7% w.v.<sup>2</sup> added to sample).

Temperature:

Chamber, 41° C. (v.p.<sup>3</sup> = 57.98 mm.).

Absorption tubes, 32.5° C. (v.p. = 36.4 mm.).

Barometer, 759 mm.

Iodine reduced, 50.25 cc. (N.F. = 0.00093).

$759 - 36.4$  (v.p. in absorption tubes) = 722.6 mm. pressure.

$\frac{722.6}{759} \times 5,990 = 5,703$  (cc. gas mixture in sample).

$5,990 - 5,703 = 287$  (cc. w.v. at 100% humidity).

$287 \times 0.617$  (61.7%) = 177.18 cc. (w.v. added to sample outside chamber).

$5,990 - 177.1 = 5,812.9$  (cc. in sample at 32.5° C., 759 mm.).

$759 - [38.3 \times 57.98$  (v.p. at 41° C.)] = 736.8 mm. (g.p.<sup>4</sup> of sample in chamber).

$\frac{736.8}{759} \times \frac{273}{305.5} \times 5,812.9 = 5,042.5$  (vol. in cc. of sample minus SO<sub>2</sub> removed).

Iodine reduced = 50.25 cc. with N.F. of 0.00093

= 0.0467 cc. normal iodine reduced.

(1 cc. normal iodine = 0.0112 cc. sulfur dioxide (s.c.<sup>5</sup>).

$0.0467 \times 0.0112 = 0.5234$  (cc. sulfur dioxide in sample).

$5042.5 + 0.5234 = 5043.2$  (cc. in total sample drawn).

$0.5234 \div 5043.2 = 0.010379\%$  SO<sub>2</sub>

= 104 parts SO<sub>2</sub> per million parts of the mixture.

#### Sources of Error

*Temperature.* A direct effect of temperature is the difficulty of keeping the water in the collecting cylinder, *l*, and in the leveling bottle, *m*, saturated with sulfur dioxide. The absorption coefficient of sulfur dioxide by water at 760 mm., according to Freese, ranges thus:

	Temp.	Liters SO <sub>2</sub>	G. SO <sub>2</sub>
I volume of water absorbs. . . . .	0°	79.79	228.29
	20°	39.37	112.90
	40°	18.78	54.11

<sup>2</sup> w.v. = water vapor.

<sup>3</sup> v.p. = vapor pressure.

<sup>4</sup> g.p. = gas pressure.

<sup>5</sup> s.c. = standard conditions.

It is obvious, from the above figures, that the sulfur dioxide capacity of water in the collecting cylinder and in the leveling bottle changes as frequently and as continuously as the temperature changes, and that re-saturation is repeatedly necessary. It was thought that a heavy mineral oil might be substituted for the water in these containers, with the hope that the solubility in such an inert substance might be very low. It was found, however, that during a period of 20 hours, 175 cc. of gas were absorbed. This rate of absorption, together with the fact that the time required to send sulfur dioxide into the case by oil-displacement was greater than the time required by water-displacement, proved the latter method the better one and it was again used. Corrections for absorption of sulfur dioxide by water are explained under "Procedure for an Experiment."

A third effect of temperature was to cause a variation in the concentration of sulfur dioxide in the chamber. This matter will be discussed in more detail under the heading "Behavior of Sulfur Dioxide in Contact with Glass."

*Rubber Stoppers.* In order to test air known to be sulfur dioxide-free it was washed by being drawn through a series of tubes containing an iodine solution, then immediately drawn through a second series similar to and directly connected to the first; if any sulfur dioxide was present in the sample of air, the iodine in the first series of tubes absorbed it, and there could be no gas present to reduce iodine in the second series; the iodine of each series was titrated separately.

TABLE I. *Tests of Outside Air (April 2)*

Trial	Cc. I <sub>2</sub> Used	Cc. I <sub>2</sub> Reduced	Min. for Drawing Sample	Cc. H <sub>2</sub> O for Sample	Temp. °C.	Cc. SO <sub>2</sub> Found	SO <sub>2</sub> Found (Parts in Million)
I {	a...	25.70	13	8,675	41.5	0.1652	19
	b...	24.935				0.1460	17
II {	a...	24.96	6.75	8,640	41.5	0.0448	5
	b...	24.98				0.0581	6.7
III {	a...	24.95	6	8,635	41	0.0918	10.6
	b...	25.00				0.0786	9
IV {	a...	24.96	21	8,120	39	0.1400	17
	b...	24.94				0.1341	16.5
V {	a...	24.99	21.5	8,160	38.5	0.1145	14.4
	b...	25.00				0.1445	17.7

*Note.* Six tubes were used in each set of trials: two series (a and b), each containing two tubes with iodine and one with potassium iodid.

Such tests showed that (a) the reduction of iodine in the second series of tubes in which the sample was known to be free of sulfur dioxide was as great as in the first series, and (b) the time used in drawing the sample had a relation to the quantity of iodine reduced.

At this stage of the investigations rubber stoppers were used for the absorption tubes, and it was thought that the loss of iodine which appeared to show the presence of sulfur dioxide was due to reduction by the rubber, although the rubber stoppers were fully ten centimeters from the solution. The idea was strengthened by the fact that there was an increase in the quantity of iodine reduced as the length of time for drawing the sample increased. A careful study of this point was made by making tests with outside air after having coated all rubber stoppers and connecting tubing with paraffin.

TABLE 2. *Rubber Stoppers and Tubing Coated with Paraffin (April 3)*

Trial	Cc. I <sub>2</sub> Used	Cc. I <sub>2</sub> — Equiv. of Titration	Min. for Drawing Sample	Cc. H <sub>2</sub> O for Sample	Temp. ° C.	SO <sub>2</sub> — Found (cc.)	SO <sub>2</sub> — Found (%)
I { a...	24.95	26.00	13	8,450	51.5	*Slight neg. value	Slight neg. value
b...	24.90	26.82					
II { a...	25.00	27.44	9.3	8,610	48.5	Slight neg. value	Slight neg. value
b...	24.94	24.95					
III { a...	25.00	28.31	8	8,610	45.5	Slight neg. value	Slight neg. value
b...	24.97	28.28					

\* Negative value is shown in that the iodine used is slightly less in quantity than the iodine-equivalent of titration. These negative values were later eliminated when neither distilled water nor potassium iodide was added to the iodine before titration. See discussion of this point under the heading "Variations in Solutions."

Table 2 shows that when rubber surfaces were coated with paraffin there was no reduction of iodine, and the fact is obvious that rubber had been reducing the iodine solutions, thus seeming to indicate the presence of sulfur dioxide. It was clear that the use of rubber must be eliminated, and therefore absorption tubes with ground-glass stoppers were used.

*Variations in Solutions.* In the early determinations for sulfur dioxide content, potassium iodide, instead of starch, was used in the last absorption tube; after the sample had been drawn through the solutions, both iodine and potassium iodide were poured into a beaker, and, in order to prevent loss of iodine, the tubes were carefully washed with distilled water or with potassium iodide; the titration of this diluted iodine was then carried out in the beaker. It was found that such dilution of iodine had an effect upon the relation of the sodium thiosulfate solution to the iodine solution, which relation varied as the quantities of distilled water and of potassium

iodid varied. It was therefore necessary to avoid these variations by replacing the potassium iodid in the third absorption tube by starch, and to carry out titrations directly in the absorption tubes; the ground-glass stopper of the absorption tube (see text fig. 4) was devised at this point.

A variation was also found in the ratio of the sodium thiosulfate to the iodine solution, which ratio decreased slightly from day to day. The following results are typical, and show that it was necessary daily to find the relation between the solutions:

June 20,  $\frac{\text{Na}_2\text{S}_2\text{O}_3}{\text{I}_2}$  relation, 0.9878.

June 21, 0.9600.

June 23, 0.9568.

June 24, 0.9555.

June 25, 0.9507.

### Accuracy of Method

The method is proven to be accurate to one part in a million and fairly accurate to two parts in ten million; the range of error is five parts in ten million.

The accuracy of the method, as found in blank tests, is shown in table 3.

TABLE 3. *Accuracy of Method (May 12)*

Trial	Cc. I <sub>2</sub> Used	Cc. I <sub>2</sub> Reduced	Min. for Drawing Sample	Cc. H <sub>2</sub> O for Sample	Cc. I <sub>2</sub> Error	SO <sub>2</sub> — Equiv. of Error (Pts. per Million)	
I {	a....	24.93	24.964	7	5,800	+0.066	+0.123
	b....	24.85	24.852			-0.002	-0.004
II {	a....	24.95	25.128	7	5,790	-0.178	-0.331
	b....	24.96	24.864			+0.096	+0.178

After the accuracy in the method of making determinations was established, it seemed desirable to determine the purity of the sulfur dioxide, as well as the degree of accuracy obtained in measuring the gas in the collecting cylinder. With these aims in mind, the absorption tubes, containing the iodine solution, were connected to the collecting cylinder, and the sample was sent directly from the collecting cylinder into iodine.

TABLE 4. *Purity and Accuracy in Quantity of Sulfur Dioxide (May 13)*

Trial	Cc. Sample SO <sub>2</sub> * (Stand- ard Condi- tions)	Cc. I <sub>2</sub> Used	Cc. I <sub>2</sub> Reduced	Temp. ° C.	Cc. SO <sub>2</sub> Determined	Cc. SO <sub>2</sub> Error
I....	38.20	47.76	34.52	20	38.28	0.08
II....	42.77	42.30	36.58	28	40.84	1.93

\* No correction for vapor pressure.



The discrepancy shown between the quantity of sulfur dioxide in the sample and the quantity determined is within the limit of error in reading the volume of sulfur dioxide. As shown later in this paper, the quantity of gas sent into the chamber differs very much from the quantity found present in the chamber, and it is therefore not essential that the method of measuring the pure sulfur dioxide be absolutely accurate. On the other hand, it is very important that the method of determining the concentration of gas present have the greatest degree of accuracy.

#### BEHAVIOR OF SULFUR DIOXIDE IN CONTACT WITH GLASS

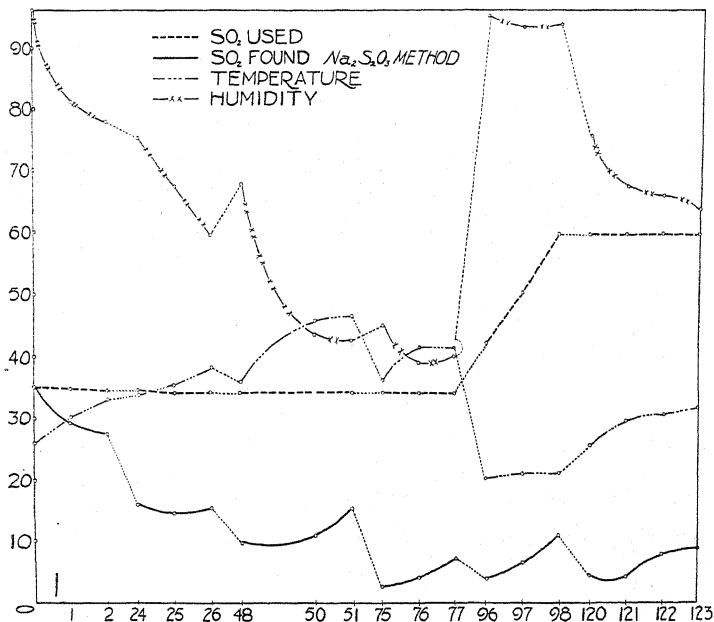
Interesting phases of the behavior of sulfur dioxide were presented in (a) the wide discrepancy between the quantity of gas sent into the chamber and the quantity determined immediately afterward; this difference is called the sulfur dioxide discrepancy; (b) the less rapid but continuous disappearance or loss of sulfur dioxide, and (c) the fluctuation in sulfur dioxide concentration with changes in temperature.

The relatively small amounts of sulfur dioxide determined immediately after a considerable quantity of gas had been sent into the chamber, and a further apparent loss or disappearance of a large proportion of the gas, an hour later, led to repeated checks on the accuracy of the method. The problem was first approached from the standpoint of possible leakage from the chamber. In order to determine whether this was a possible cause of the loss of sulfur dioxide, it was sent in large quantities from the collecting cylinder into a 19-liter carboy, from which the leakage was negligible. The carboy was well shaken immediately before a determination, so as to insure mixing of the gas. The results obtained were but slightly more uniform than those obtained from the chamber. After repeated determinations having similar results, it was certain that the leakage of gas from the chamber was not the causal factor of the sulfur dioxide discrepancy, or of the later disappearance of the gas.

A careful study of this behavior, with repeated experiments in the carboy and in the chamber, showed that the adsorption of sulfur dioxide on surfaces, and oxidation, both being influenced by temperature, and perhaps other possibilities, are the controlling factors in regulating the sulfur dioxide content of the air-gas mixture. The sulfur dioxide discrepancy was often as much as 75% of the gas used (see fig. 3), and its further gradual loss, with duration of time, was such that a second test, one hour after the first, frequently showed but 50% of the first determination. Fluctuations often appeared, so that certain determinations showed a higher sulfur dioxide content than previous tests in the same series, although some of the gas must have been oxidized in the meantime.

*Adsorption.* The inside walls of the glass chamber presented a large glass surface, on which the gas, upon entering the chamber, was adsorbed and inactivated, thus very considerably reducing the diffusible gas which could be drawn for analysis.

There was evidence that adsorption of sulfur dioxide gradually approached a maximum, which point may be called the "saturation equilibrium"; this was shown by the fact that the sulfur dioxide discrepancy was invariably much greater in the first determination following a period when the gas content of the chamber was low. The sulfur dioxide discrepancy decreased after large quantities of gas had been used for a number of consecutive days, but saturation of the glass was apparently difficult to obtain, and was of short duration (see text figs. 1, 2, 3).

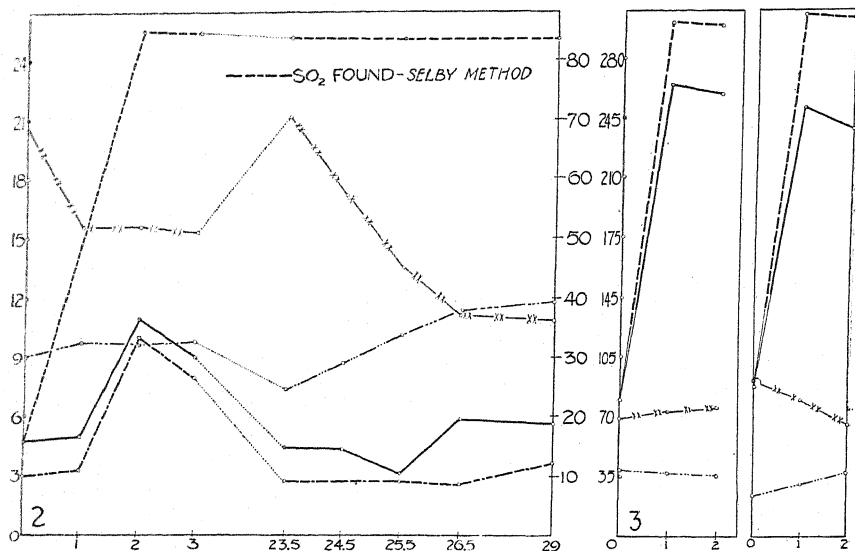


TEXT FIG. 1. Discrepancy, loss, and fluctuations of sulfur dioxide, June 23-28. Ordinates: sulfur dioxide in cc.; temperature in degrees C., and humidity in percentages. Abscissae: time in hours. . . ., interval of time during which no analyses were made.

The sulfur dioxide discrepancy decreased markedly when glass surfaces were eliminated by having the inside walls of the chamber coated with paraffin.

*Oxidation of Sulfur Dioxid to Sulfuric Acid.* Besides the loss of sulfur dioxide due to adsorption, there was a further loss due to oxidation from sulfur dioxide to sulfur trioxide. This was proved by the formation of a precipitate of barium sulfate from a neutral solution of barium chlorid put into the chamber; the precipitate was shown to be barium sulfate, and not barium carbonate, by its insolubility in nitric acid. The beaker containing the barium chlorid had been coated with paraffin, so that oxidation by catalysis was reduced to a minimum. The process of oxidation was found to be less rapid after the inside of the chamber was coated with paraffin,

indicating that oxidation, as well as adsorption, is less active in contact with a paraffin than with a glass surface.



TEXT FIG. 2. Comparison of  $\text{Na}_2\text{S}_2\text{O}_3$  and Selby methods, July 2, 3. Ordinates to left of figures, sulfur dioxide in cc.; ordinates to right of figures, temperature in degrees C. and humidity in percentages. Abscissae and . . . as in text fig. 1. The lowest curve is for the Selby method. Explanation of other curves as in text fig. 1. TEXT FIG. 3. Discrepancy and loss of sulfur dioxide, June 20, 21. Ordinates, abscissae, and . . . as in text fig. 1. Explanation of curves as in text fig. 1.

TABLE 5. Results for Glass Surface (May 2)

Trial	Temp.	Cc. $\text{SO}_2$ Used	Cc. $\text{SO}_2$ Determined	% $\text{SO}_2$ Determined	% $\text{SO}_2$ Lost
I....	25.6	268.75	174.68	65	35
II....	24	none	139.66	52*	48

Results for Paraffin Surface (June 18)

I....	32.3	227.0	223	98	2
II....	30.4	none	195	85†	15

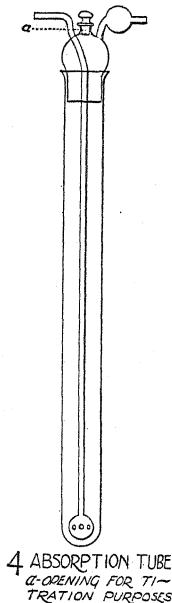
Note. The higher temperature of June 18 tends to give a somewhat higher percentage in concentration, but it does not account for the total increase over May 2; the greater increase is due to the less rapid rate of adsorption and of oxidation in contact with a paraffin than with a glass surface.

\* 80% of trial I.

† 86.7% of trial I.

Temperature and humidity are important factors influencing the adsorption and the oxidation processes discussed above, and therefore have

a great effect in determining the percentage of sulfur dioxide obtained in an analysis. A study of the fluctuations, which frequently showed by a higher sulfur dioxide content in certain tests than in previous ones of the same series, proved that at high temperature and low humidity there is less



TEXT FIG. 4. Absorption tube  $\times$  about  $\frac{1}{4}$ . Closely fitting ground-glass stopper has three openings: (1) inlet, (2) opening, *a*, for titration purposes, and (3) outlet. Length of tube, 45 cm.; outside diameter, 3.4 cm.; span of arms, 10.5 cm.

adsorption on surfaces and a less rapid rate of oxidation than at low temperature and high humidity (see text figs. 1, 2, 3). A coat of lime on the top and outer sides of the chamber, to diffuse the sunlight, reduced the range in temperature variation and therefore the range of fluctuation in concentration of the gas.

#### COMPARISON OF THE METHOD DEVELOPED WITH THE METHOD USED BY THE SELBY SMELTER COMMISSION

The method used by the Selby Smelter Commission in California was compared with the method herein discussed and was found to be less accurate. The report of this commission, published by the Bureau of Mines in 1915, describes their method which consisted primarily in the use of two 24-liter aspirating bottles, each containing 250 cc. of fresh starch solution; one aspirator was evacuated to a 380-mm. vacuum; the other one, not evacuated, was used for a "blank" or control determination. The sample of sulfur dioxide-air mixture was taken by opening a stopcock of the evacuated aspirator and allowing the mixture to rush into the flask,

thereby bringing the pressure within the aspirator back to atmospheric pressure. After both aspirators had been shaken vigorously so as to have the sulfur dioxide of the sample absorbed by the iodine, the solution from each aspirator was transferred to a 500-cc. test bottle; the colors of the solutions were compared, and sufficient 0.002 *N* iodine solution was added to the solution containing the sample to bring the color to that of the control. The quantity of iodine needed to obtain the required color represents the quantity reduced by the sulfur dioxide, and with these figures the gas content of the volume drawn into the aspirator was calculated.

Plate IX shows the arrangement of apparatus as used in these comparative determinations. The outlet, *u*, from the gas chamber is connected to both the aspirator, *w*, and the absorption tubes, *x*, by means of the three-way stopcock, *v*; the aspirator, evacuated to 380 mm., receives the sample for determination according to the Selby method; the absorption tubes, *x*, receive the sample for determination according to the thiosulfate method. The samples are taken simultaneously, since the mixture drawn from the chamber is divided at *v* between the two arms leading respectively to the partially evacuated aspirator and to the absorption tubes. The second aspirator, *w'*, is the one containing the "blank" solution.

Many determinations, both separately and in parallel, were made with these methods for the purpose of acquiring sufficient skill in manipulation before any data or results were accepted as being significant. The comparative determinations reported were made at hourly intervals.

Comparative determinations were made with concentrations of sulfur dioxide ranging from 1.4 to 181 parts per million parts of air-gas mixture. A summary of the comparative analyses shows the following results:

Number of tests showing more SO <sub>2</sub> with Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> method	40.
Number of tests showing more SO <sub>2</sub> with Selby method	3.
Number of tests equal or doubtful <sup>6</sup> .....	2.

Text figure 2 charts the results of the comparative determinations of July 2 and July 3, and shows that determinations made with the sodium thiosulfate method indicate the presence of more sulfur dioxide than those made with the Selby method. From the results of these comparisons it seems certain that some of the sulfur dioxide is lost when determinations, in very dilute mixtures of the gas, are made according to the Selby method. The percentage of this error probably increases with dilution, since the quantity of sulfur dioxide lost on the large surface of the aspirator represents a greater percentage of loss in small quantities of sulfur dioxide than in large quantities of the gas.

It is reasonable to believe that the Selby method offers probable sources of error in (a) failing to correct for vapor pressure in the sample taken,

<sup>6</sup> Selby results higher than sodium thiosulfate if former are corrected for vapor pressure.  
Selby results lower than sodium thiosulfate if former are not corrected for vapor pressure.

TABLE 6. *Comparative Tests Made by Sodium Thiosulfate and Selby Methods*

Trial	Method	Temp. (° C.)	Humid. (%)	Barom. (mm.)	% SO <sub>2</sub> Determined	Parts SO <sub>2</sub> per Mill.
June 23	I.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> * <i>b</i> †	26	96.4	762	0.002183	21.8
		26	96.4	762	0.002095	21.0
		26	96.4	762	0.002022	20.2
	II.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	30	81.0	762	0.001808	18.0
		30	81.0	762	0.001968	19.7
		30	81.0	762	0.001883	18.8
	III.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	33	77.7	762	0.001697	17.0
		33	77.7	762	0.001639	16.4
		33	77.7	762	0.001557	15.6
June 24	I.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	33.9	75.0	761	0.000983	9.8
		33.9	75.0	761	0.000749	7.5
		33.9	75.0	761	0.000713	7.1
	II.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	35.3	67.2	761	0.000885	8.9
		35.3	67.2	761	0.000841	8.4
		35.3	67.2	761	0.000796	8.0
	III.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	38.3	59.2	761	0.000942	9.4
		38.3	59.2	761	0.000743	7.4
		38.3	59.2	761	0.000699	7.0
June 25	I.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	35.9	67.8	758.5	0.000599	6.0
		35.9	67.8	758.5	0.000487	4.9
		35.9	67.8	758.5	0.000460	4.6
	II.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	45.5	43.3	756	0.000651	6.5
		45.5	43.3	756	0.000530	5.3
		45.5	43.3	756	0.000483	4.8
	III.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	46.4	42.2	756	0.000941	9.4
		46.4	42.2	756	0.000605	6.1
		46.4	42.2	756	0.000437	4.4
June 26	I.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	36	44.7	761.5	0.0001430	1.43
		36	44.7	761.5	0.0000106	0.11
		36	44.7	761.5	0.0000100	0.10
	II.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	41	38.6	761	0.0002360	2.36
		41	38.6	761	0.0000638	0.64
		41	38.6	761	0.0000593	0.59
	III.. Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> Selby <i>a</i> <i>b</i>	41	39.7	761	0.0004275	4.28
		41	39.7	761	0.0001463	1.46
		41	39.7	761	0.0001359	1.36

\* Vapor-pressure corrections made in tests *a*.† Vapor-pressure corrections not made in tests *b*.

(b) difficulty in maintaining a partial vacuum in the aspirator, (c) water vapor in the aspirator probably not acting according to Boyle's law, (d) rubber stopper, used in the neck of the aspirator, reducing the iodine, and (e) loss of sulfur dioxide and iodine on the large glass surface of the aspirator.

#### SUMMARY

In studying the effects of sulfur dioxide upon vegetation it was found that methods of determination of the low concentrations of gas causing minimal injury to plants were unsatisfactory. Any method for this purpose may be subject to errors because the gas is invisible, extreme dilutions must be used, changes in temperature cause changes in volume, the gas is adsorbed on surfaces, and oxidation from sulfur dioxide to sulfur trioxide is relatively rapid.

The investigations indicate a point of general interest with reference to effects of the gas upon vegetation near industrial plants which emit sulfur dioxide from smokestacks. The relatively rapid oxidation of sulfur dioxide to sulfur trioxide confines the former to a rather small radius, limiting liability to injury to a more reduced area than is sometimes supposed; consequently the damage done to vegetation is likely to be very slight.

#### CONCLUSIONS

1. Methods of burning sulfur for experimental purposes are unsatisfactory, because of the production of sulfur trioxide and of sublimed sulfur.
2. Use of alcohol for the purpose of supplying heat, or of mixing with carbon bisulfide, is likely to result in the production of acetaldehyde. A chemical method is the most satisfactory one for obtaining the gas. Pure sulfur dioxide may be obtained from sodium bisulfite by the use of the method and apparatus here described.
3. Determinations of the concentration of sulfur dioxide at close intervals (15- or 20-minute intervals) is necessary because of the instability of the gas.
4. Decrease of the percentage of sulfur dioxide was found to be caused by absorption by plants and soil, adsorption on surfaces, oxidation from sulfur dioxide to sulfur trioxide, and probably other possibilities.
5. Oxidation from sulfur dioxide to sulfur trioxide is relatively rapid.
6. Adsorption and oxidation were found to be less active (a) in low temperatures than in high ones, so that higher percentages of sulfur dioxide were determined in high temperatures than in low ones; (b) in contact with paraffin than with glass surfaces. therefore the inside surface of the gas chamber was coated with paraffin; (c) as the degree of saturation of surfaces increased.
7. Rubber reduced an iodine solution used for determining the concentration of sulfur dioxide, resulting in an error in the determinations. This was found to be true though the rubber was not in contact with the solution. Therefore rubber stoppers may not be used in an analysis of the gas.

8. The content of the gas chamber was analyzed by drawing a sample of the mixture through an iodine solution in a series of absorption tubes with ground-glass stoppers, adapted for titration of excess iodine *in situ*, with a sodium thiosulfate solution.

9. The method developed was compared with that used by the Selby Smelter Commission in 1915, and was found to be more accurate for determining sulfur dioxide in dilutions needed for minimal injury to plants.

10. The advantages of the method are believed to be the following: (a) the glass surface, on which sulfur dioxide may be lost, is reduced to a minimum; (b) elimination of rubber near an iodine solution avoids reduction of iodine by that medium; (c) the method corrects for vapor pressure—a correction not made in previous methods.

11. The method is believed to be accurate to one part of sulfur dioxide in a million parts of air-gas mixture, and fairly accurate to two parts in ten million.

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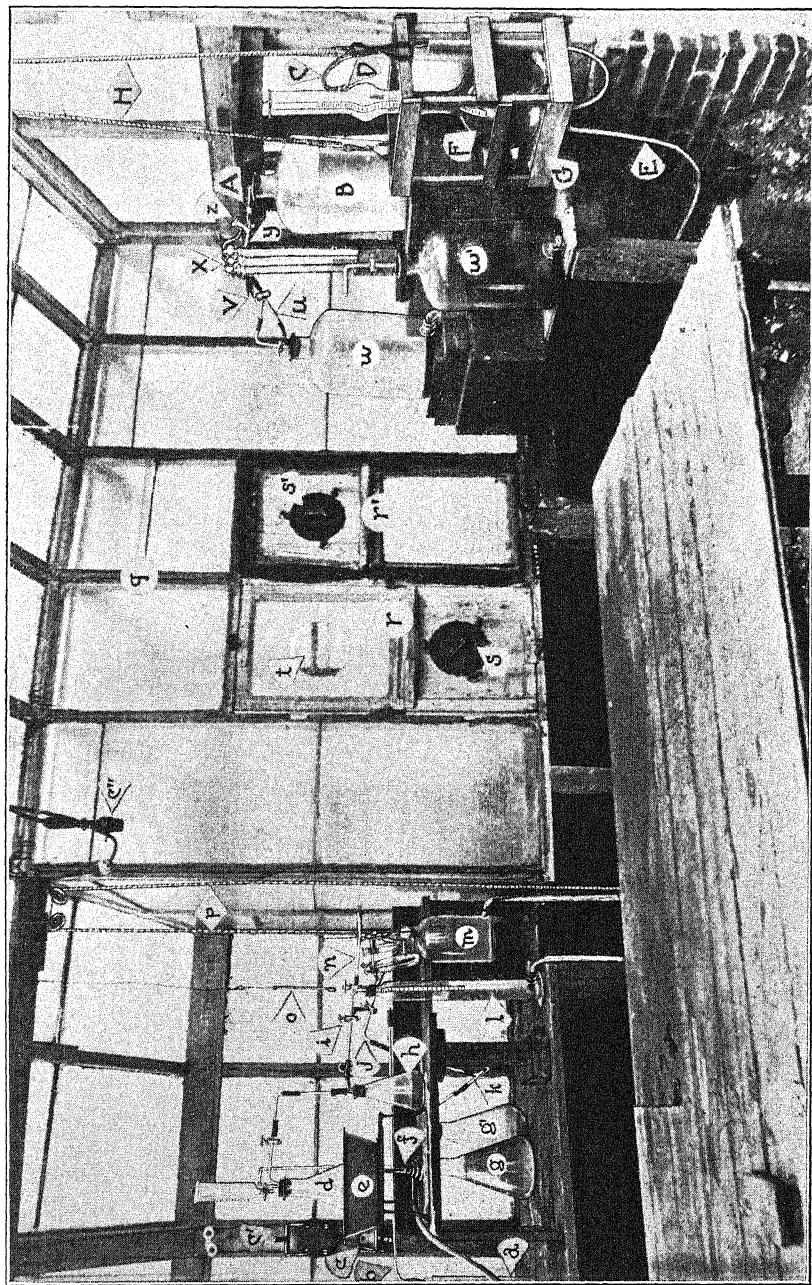
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### EXPLANATION OF PLATE IX

#### *Equipment for Experiments with Sulfur Dioxid*

- a*, cylinder containing sodium hydroxid for testing sulfur dioxid.
- b*, tube to conduct sulfur dioxid from washing bottle, *h*, to testing cylinder, *a*.
- c*, *c'*, electric switches; *c* to motor for fan in gas chamber, *q*; *c'* to vacuum pump used for Selby method.
- c''*, wire connection and socket for vacuum pump.
- d*, sulfur dioxid generator, fitted with (1) separatory funnel for sulfuric acid, (2) outlet connected to aërating bulb in washing bottle, *h*, (3) outlet to first equilibrium flask, *g*.
- e*, water bath.
- f*, Bunsen burner.
- g*, *g'*, equilibrium flasks to control gas pressure in generator.
- h*, washing bottle containing distilled water saturated with sodium bisulfite and sulfur dioxid. Fitted with an inlet tube terminated with an aërating bulb, and with an outlet tube connected to the three-way stopcock, *i*.
- i*, three-way stopcock from washing bottle, *h*, to collecting cylinder, *l*, and to a second three-way stopcock (seen at back of *i*). The second stopcock leads to tube *b* and to tube *j*, connected to the Y-tube, *k*.
- j*, tube from second three-way stopcock to one arm of the Y-tube, *k*.
- k*, Y-tube leading out of doors; one arm is connected to *j* from the second three-way stopcock (see *i*); the other arm is connected to the second equilibrium flask, *g'*.
- l*, sulfur dioxid-collecting cylinder, calibrated in cc., and containing distilled water saturated with sodium bisulfite and sulfur dioxid; three openings: (1) inlet from three-way stopcock, *i*, (2) outlet, *n*, to gas chamber, *q*, and (3) outlet at bottom connected to leveling bottle, *m*.
- m*, leveling bottle, connected to collecting cylinder, *l*; it contains distilled water saturated with sodium bisulfite and sulfur dioxid. Two openings at the top: (1) vertical tube to carry and control rise of water due to sudden flow of gas into the collecting cylinder, *l*; (2) outlet to two U-tubes in series. The first U-tube contains mercury, the second sodium hydroxid.



WEIERBACH: EFFECTS OF SULFUR DIOXID



- n*, outlet tube with stopcock, from collecting cylinder, *l*, to chamber, *g*.  
*o*, thermometer to determine temperature of sulfur dioxide when sent into chamber.  
*p*, rope-and-pulley system; weight attached to rope is not shown in the plate.  
*q*, gas chamber in which experimentation was carried on. Electric fan and wet-and-dry bulb psychrometer are within the chamber.  
*r, r'*, closely-fitting doors: *r*, unsealed, so that it may be removed; *r'*, sealed.  
*s, s'*, removable discs closely fitting into openings, which are further sealed with sheet rubber.  
*t*, space cleared of paraffin coating on inner surface, for window purposes.  
*u*, outlet from gas chamber to three-way stopcock, *v*.  
*v*, three-way stopcock from chamber, *g*, to semi-evacuated aspirator, *w*, and to absorption tubes, *x*.  
*w*, 12-liter aspirator containing starch iodide solution; evacuated to 380 mm. pressure.  
*w'*, control, or "blank" aspirator, containing starch iodide solution at 760 mm. pressure.  
*x*, series of three absorption tubes (see text fig. 4). The first two tubes contain iodine solution, the third contains starch solution.  
*y*, tube (with stopcock not shown) from stopcock, *v*, to T-tube, *A*, leading to the aspirator, *B*; *y* is used to wash air out of the system.  
*z*, stopcock between the absorption tubes, *x*, and the T-tube, *A*.  
*A*, T-tube from wash tube, *y*, and from absorption tubes, *x*, to the aspirator, *B*.  
*B*, aspirator containing water which is tapped in order to draw the sample of gas mixture from the chamber. Inlet at top from T-tube, *A*; two outlets below: (1) to tube *C*, (2) to tube *E*.  
*C*, rubber tube connecting aspirator, *B*, to measuring flask, *F*.  
*D*, lip on bulb for inlet tube connected to the aspirator.  
*E*, outlet from the aspirator, for water not measured; used when washing air from the system.  
*F*, measuring flask, calibrated in cc.; tall neck, with bulb, for leveling purposes; the inlet tube, from *D*, reaches to the bottom of the flask.  
*G*, crate for *F*.  
*H*, rope-and-pulley system connecting crate, *G*, to weight (not shown in the plate) for the purpose of moving the measuring flask.

# INTERNAL DECLINE OF LEMONS III. WATER DEFICIT IN LEMON FRUITS CAUSED BY EXCESSIVE LEAF EVAPORATION<sup>1</sup>

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## INTRODUCTION

This paper presents a continuation of the studies reported in a previous paper (3) on the physiological disease known as internal decline or endoxerosis<sup>2</sup> of lemon fruits. A full description of the disease may be found in a former paper of this series (2).

It is a common observation that, of the shoots pruned from lemon trees, those which bear fruits do not wilt as rapidly as those not bearing fruits. It has also been noted that the lemons on the pruned-off branches become soft and flaccid much more quickly than those fruits which merely fall from the trees. Furthermore, during the afternoon of a hot, dry day those lemons which remain attached to the tree become comparatively soft while by early the next morning they have regained their turgidity.

These observations indicate that excessive leaf evaporation may cause a water deficit in the fruit, since, at times when the roots and the intervening water-conducting system can not supply water to the leaves as rapidly as it is evaporated, the fruits act as reservoirs which may be drawn upon to overcome partially at least the deficit in the leaves. That such is the case has been known for many years, but the extent and full importance of this fact with reference to the development of certain fruits is not fully appreciated.

## HISTORICAL

As early as 1729 Langley (10) wrote:

'Tis very easy to conceive, that if very dry Weather exhales away that Moisture which is necessary for those Formations (The internal parts of the fruit, such as the kernels, stones and the like) the Work will be imperfect and consequently the Fruits must perish.

In this case Langley was referring to the excessive dropping of young fruits, but his statement shows that even at that early date it was recognized that leaf evaporation had an important bearing upon the development of fruits.

Osterwalder (15, 16) concluded that excessive shedding of blossoms and young fruits is caused largely by leaf evaporation. Chandler (4) published

<sup>1</sup> Paper no. 127, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

<sup>2</sup> The term "endoxerosis" is introduced here as a technical name for this disease.

interesting discussions and photographs showing that excessive leaf evaporation may cause a shrinkage of the fruits of the apple, cherry, plum, cucumber, gourd, watermelon, and tomato. Lloyd (11) showed that the abnormal shedding of young cotton bolls is due to excessive evaporation, and Heinicke (7) concluded that excessive dropping of young apples may be largely caused by the same factor. Hodgson (8) reported experiments which showed that during periods of excessive leaf evaporation orange fruits decrease in size. MacDougal (12) made a direct measurement, by the use of a dendrograph, of tomato fruits as affected by excessive leaf evaporation. He says:

These watery fruits showed in a very marked manner the effect of water loss or transpiration on the growth of the fruits. As the daily temperature of the fruits rose from 12° and 14° C. to 26° and 28° C. acceleration ensued up to a point where the rise caused a water loss overbalancing the gain by hydration. It was not possible to increase the water supply by watering the soil around the roots in such a manner as to cancel the mid-day shrinkage or slackening in growth at other times.

In a more recent article, MacDougal (13) has reported some further interesting results concerning the behavior of the walnut during the daily periods of excessive evaporation. He found that the nut begins to decrease in diameter about the middle of the forenoon and that it does not again reach its early forenoon diameter until about 3 or 4 P.M. The decrease in diameter corresponds to the period of greatest stomatal opening, which on foggy days is greatly diminished.

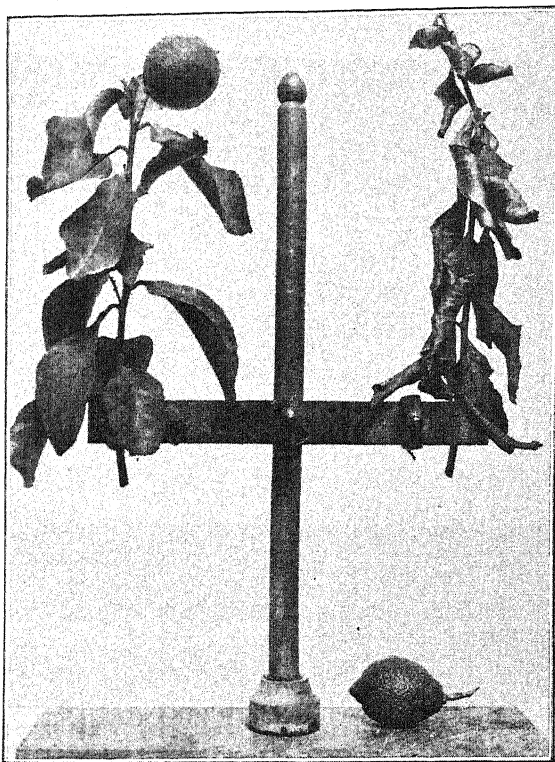
Further discussion bearing directly or indirectly on the subject of withdrawal of water from the fruits by the leaves may be found in articles by Renner (19), Livingston and Brown (9), Coit and Hodgson (5), Radspinner (17), Gadd (6), and others.

#### METHODS AND RESULTS OF EXPERIMENTATION

Text figure 1 shows very clearly the extent to which lemon fruits may act as water reservoirs for the leaves. Lemon branches were brought to the laboratory, fastened in an upright position, and allowed to remain in this position in the laboratory for several days. Photographs were taken at intervals. Figure 1 shows the conditions of the branches at the end of 68 hours. The maximum and minimum laboratory temperatures for the four days concerned were 86, 86, 88, and 84° F., and 73, 72, 75, and 73° F., respectively. Relative humidities were not taken in the laboratory, but outside, near the buildings, they were 25, 25, 41, and 38 percent at noon of the respective days. The detached lemons remained comparatively firm, while the attached ones became noticeably less turgid. When this photograph was taken, the leaves on the branch from which the lemons had been detached were dry enough partially to crumble in the hand.

The shrinkage of, and water lost from, these particular lemons were not determined. Tests on other lemons, however, showed (a) that during

the first 48 hours attached lemons decreased in diameter from two to three times as much as the detached fruits, and (b) that during the same period the attached lemons lost from 25 to 35% more water than the detached ones.

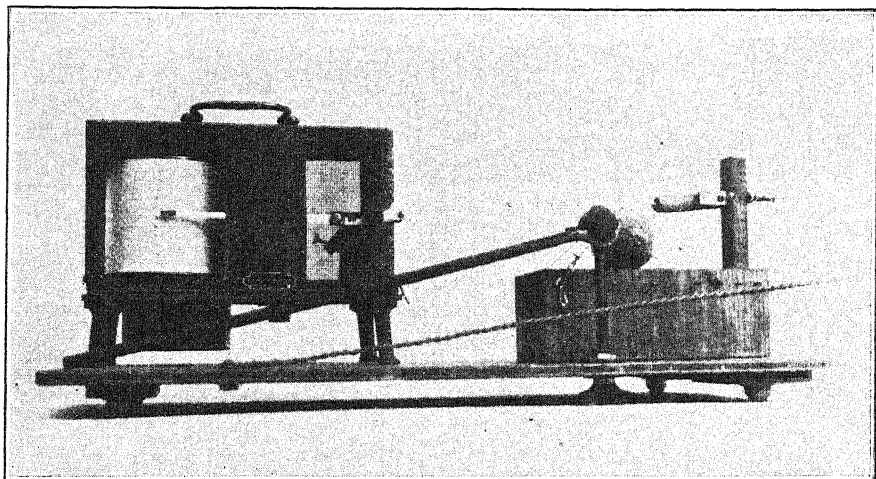


TEXT FIG. 1. Extent to which lemon fruits may act as water reservoirs for the leaves. Photograph taken 68 hours after bringing the branches to the laboratory. Branches were fastened to the support as shown, and the support was allowed to stand on the laboratory table during the entire 68-hour period.

Because of the importance of the withdrawal of water from the fruits, a special auxograph was devised for its study. It was hoped that with the use of an auxograph information might be obtained concerning the time of day when water withdrawal begins and ends, the effect of climatic factors, and other influencing conditions.

The instrument is very simple and has proved very satisfactory for the purpose at hand. It consists of a partially dismantled thermograph to which a few very simple attachments have been added (text fig. 2). As shown in the figure, the lemon rests on a block of wood. This block is of oak, so cut that the long axes of the wood cells are perpendicular to the platform upon which it rests. The block was thoroughly impregnated with

boiling paraffin. The coefficients of expansion of the block and of the base of the thermograph were practically the same, so that no appreciable difference could be detected while the apparatus was in operation to test this particular point.



TEXT FIG. 2. Auxograph: instrument used to measure the diurnal contraction and expansion of lemon fruits, caused by withdrawal of water by the leaves during the day and its re-entrance during the night. When in operation, the jointed iron rod and the twisted wire swing into the foreground and are attached to the branch.

Reference to text figure 2 will show that a change in the diameter of the lemon is transmitted to the pen through the use of two levers. When evaporation begins, the lemon, if water is withdrawn from it, naturally shrinks, the lever resting upon it drops a commensurate amount, and the pen moves upward. The graphs were not augmented, in consequence of temperature or moisture expansion in the apparatus itself or of temperature expansion in the fruit, during either day or night. In fact the situation is just the reverse, there having been a slight deduction rather than an augmentation. While this deduction should be borne in mind in observing the graphs, by referring to text figure 3 it will be seen that its importance is only minor.

The graph shown in text figure 3 was obtained by the use of a detached lemon that had been very lightly coated with low-melting-point paraffin in order to prevent surface evaporation. That the fluctuations of the pen as caused by apparatus expansions or contractions are almost negligible is shown by the fact that this graph is almost a straight line. The pen reached its lowest point about 4 or 5 P.M. and its highest point about 7 or 8 A.M., a condition which is just the opposite of that shown in text figures 4 and 5. The agreements are not always exact, because of other influencing factors which will be mentioned in later discussions.



The differences between maximum and minimum temperatures during the time this test was being made ranged from 41 to 45° F.

The auxographs have been calibrated so that a given distance of movement of the pen on the chart may be translated into terms of lemon-expansion or -shrinkage. For example, to cause the pen to ascend 15 mm. means that, barring corrections, the lemon has decreased in diameter 0.5 mm., provided it acted as a fulcrum under the outer lever at the point designated at number 1, 0.6 mm. if under number 2, 0.7 mm. if under number 3, and so on. (See text fig. 2.)

In order to obtain information concerning the effect of such factors as excessive leaf evaporation, it was obviously necessary to attach the auxograph to the lemon while the latter was still attached to the branch. This necessitated a provision for preventing irregular movement of the pen such as might be caused by accidental jarring of the apparatus or by wind disturbances. The desired end was very satisfactorily accomplished by placing the apparatus upon a light-weight platform having ball-bearing casters, which in turn rested on glass plates. The platform was anchored to the branch bearing the fruit to be measured, thus allowing any branch movement to be transmitted to the platform, which in turn produced a movement of the apparatus as a whole rather than of any one of its units.

When in operation the auxograph was placed in a standard thermograph shelter, the door of which was removed and hinged at the top instead of at the bottom. A branch bearing a lemon was allowed to project into the shelter, and the lemon was attached to the auxograph. This arrangement prevented exposure of the lemon and apparatus to direct sunlight or to rain.

In considering the graphs it should be remembered that *the pen ascends on the chart as the lemon shrinks and descends as it expands, hence the peak of the curve denotes time of maximum water deficit in the lemon and the depression indicates the time of its greatest turgidity.*

Changes in the water content and rates and total growth of lemons under different climatic and soil-moisture conditions are shown in the following auxographic records. The lemons used were almost but not quite mature.

*Records 7 a to d.* On July 9, nine days after an irrigation, a lemon was placed under the auxograph lever. For the first few days the diurnal fluctuations of the pen amounted on an average to about 24 mm. (text fig. 4). However, by the end of the week the magnitude of the fluctuations began to increase and continued to increase during the next 15 days (text figs. 5, 6, 8), or until the time of the next irrigation, July 30, when the fluctuations had reached a maximum of 74 mm.

Computations from previous calibrations showed that the 24-mm. diurnal fluctuation of the pen (text fig. 4) for the first few days was caused by a diameter shrinkage in the lemon of 0.96 mm., while the fluctuations of

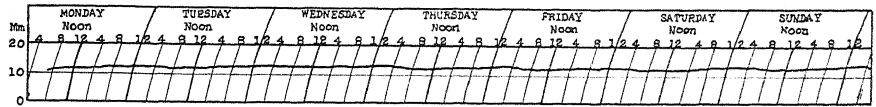


Figure 3

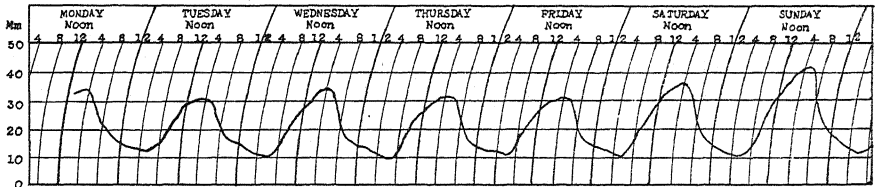


Figure 4

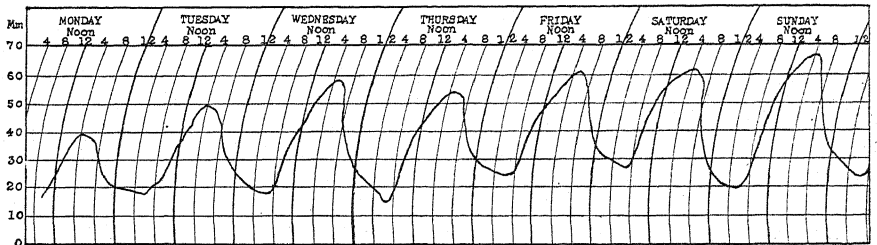


Figure 5

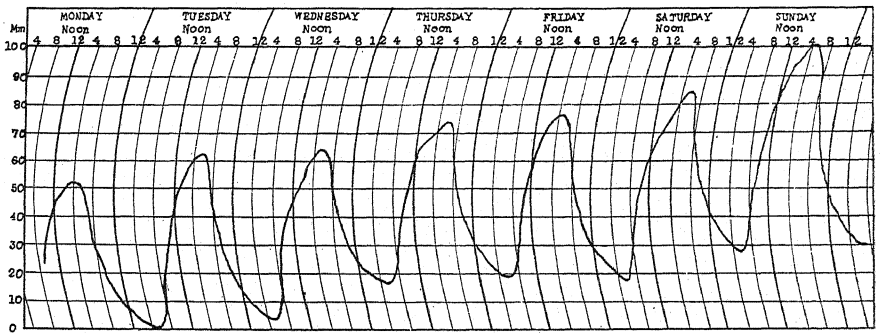


Figure 6

TEXT FIG. 3. Auxographic record made by a detached lemon that had been lightly coated with paraffin to prevent surface evaporation. This record shows that diurnal fluctuations in the graph, due to expansions or contractions of the apparatus or lemon as caused by changes in temperature, are very small. TEXT FIG. 4. Auxographic record made by a normal lemon during the second week following irrigation (July 9-16, 1923). Note (a) extent of diurnal fluctuation, (b) slopes of curves, which denote the rate of entrance of water into or its exit from the lemon, and (c) time in the morning at which the lemon began to shrink and in the afternoon at which it began to expand. (*The pen ascends as the lemon contracts and descends as it expands.*) TEXT FIG. 5. Auxographic record made by the same lemon that made the graph of text figure 4. Compare this graph with the previous one as directed above (a, b, c). TEXT FIG. 6. Auxographic record made by the same lemon that made the graph of text figure 4; this being the graph made just preceding an irrigation (July 23-30, 1923). Compare this graph with those of text figures 4 and 5, as directed above (a, b, c).

74 mm. (text figs. 6 and 8) just preceding irrigation indicated a shrinkage of 2.96 mm. Experimentation has shown that a decrease of 1 mm. in diameter of an average mature lemon equals a loss of about 1.6 g. of water, or about 2% of its entire water content. These figures are only approximate; they vary according to the age and composition of the lemon. By comparing text figures 4 and 6 it may be seen that (a) the total amount of water withdrawn from the lemon was very much less during the second than during the fourth week following irrigation; and (b) that in figure 4 the upward curves are not as abrupt or as nearly perpendicular as in figure 6. This indicates not only that during the week of July 9 to 16 the amount of water withdrawn from the lemon was less than during the week of July 23 to 30, but that the rate of withdrawal was less; and it also appears (c) that during the experiment (text fig. 4) the lemon began to contract at about 6 A.M. and to expand at about 4 P.M., while during the week just preceding irrigation (text fig. 6) the lemon began to contract at 6 A.M. or a little earlier, and to expand at about 6 P.M.

It is interesting to note (text fig. 8) just how long after its application was required for the irrigation water to affect the amount of water withdrawn from the lemon. The water first reached the tree at 6 P.M. on the Monday shown in text figure 8. By the second day after the application of the water the diurnal fluctuation was reduced from 74 to 35 mm. That the irrigation water had such a marked effect within this short period of time is especially interesting in view of the fact that the statement is often made that irrigation water does not have much effect upon the tree until about a week after application.

*Record 5 b.* That the amount of water lost from the surface of the lemon is small compared with that which is withdrawn from it by the rapidly evaporating leaves, is shown by the fact that when an attached growing lemon was permitted to make its auxographic record for four days and was then lightly coated with a low-melting-point paraffin the change in the magnitude of the diurnal fluctuations was very small, being 3 to 5 mm. on the chart, or about 5 to 8 percent. This agrees very well with the results obtained in an earlier experiment (3) in which this point was tested. In the former test detached lemons and lemons still attached to branches were placed in a well ventilated, electrically heated oven at 46° C. After 4½ hours a comparison of their sap concentrations was made by the freezing-point method. The increase in concentration was 1.49 atmospheres for those attached to the branches and only 0.34 atmosphere for the detached ones. This is a larger difference than was indicated by the auxograph, but it seems reasonable to conclude that at least part of this difference was due to the natural rigidity of the lemon fruit, which permitted a certain amount of water to be withdrawn without being registered by the auxograph.

The graphs shown in text figures 4, 5, 6, and 8, together with the graphs

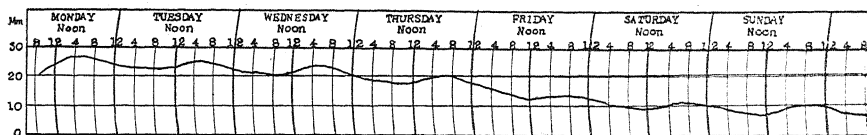


Figure 7

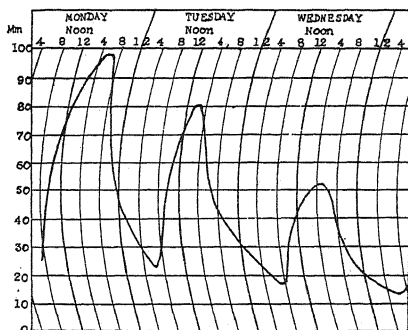


Figure 8

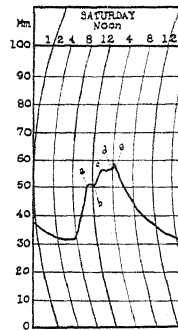


Figure 10

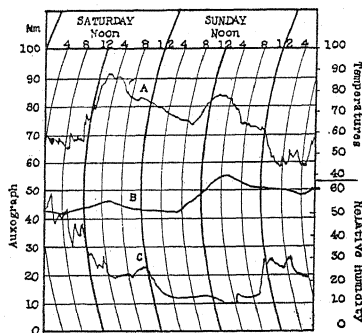


Figure 9

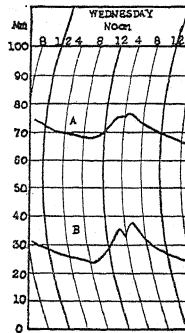


Figure 11

TEXT FIG. 7. Auxographic record made by a lemon, February 4-11, 1924. Compare the amounts of diurnal fluctuation with those shown in text figures 4, 5, and 6, made in the summer. The general downward course of the graph shows that this lemon was increasing in size from day to day, while the graphs of text figures 5 and 6 show that the lemon was gradually becoming smaller. TEXT FIG. 8. Part of auxographic record made for the week during and following irrigation (July 30 to August 6, 1923); the lemon being the one that made the graphs of text figures 4, 5, and 6. The irrigation water reached the tree at 6 P.M. Monday. Note the comparative rapidity with which the tree responded, as indicated by the amount of contraction and expansion of the lemon. TEXT FIG. 9. Comparative graphs for November 22 and 23, 1924. A, temperature; B, diurnal fluctuation in water content of lemon; C, relative humidity. TEXT FIG. 10. Auxographic record made by a lemon on a day in August when two light showers fell: *a* to *b*, effect of shower beginning at 11:00 A.M.; *b* to *c*, effect of subsequent sunshine; *c* to *d*, effect of a second shower beginning at 2:00 P.M., and intermittent cloudiness until 4:00 P.M.; *d* to *e*, effect of sunshine, which induced leaf evaporation and caused the lemon to shrink until 4:30 P.M. TEXT FIG. 11. Parts of two weekly auxographic records: A of a lemon attached to the westerly side of a tree, and B of one attached to the easterly side. The easterly half of the tree was sprayed with water for 1 hour and 10 minutes, with a westerly wind blowing. The westerly half of the tree was not sprayed, but note that the lemon on that side stopped shrinking during the time that the easterly side of the tree was being sprayed. Note. A comparison of A and B indicates that the lemon on the sprayed half of the tree (B) responded 20 to 25 minutes later than the one on the unsprayed half. The clocks were examined just before the spraying began, and it was found that this clock was too fast by just that amount. As nearly as could be judged, the two lemons began to respond at the same time.

made during the remainder of the week indicated in text figure 8 and the one for the following week, have all been joined and reproduced as a continuous graph. This graph comprising a five-weeks continuous record for a single lemon shows (a) that at the end of five weeks its diameter was actually 0.7 mm. less than when the test began, and (b) that at 6 P.M. on the evening when the irrigation water reached the tree, the diameter of the lemon was 4.5 mm. less than it was when the test began. The decrease in size of the lemon is again in agreement with the results of a previously described experiment (3) in which 200 small lemons were measured once each month until maturity. It was found that on two different occasions the average diameter measurement of the 200 lemons was less than it was a month previously.

It should be stated that the magnitude of the fluctuations shown in the graphs in text figures 4, 5, 6, and 8 is above the average, but is not an exception. The magnitude of the fluctuations is affected by a comparatively large number of factors such as general physical condition of the fruit and the tree on which it is growing, soil moisture, temperature, relative humidity, wind velocity, and sunshine. It so happened that at least most of these, and probably other factors, were in the proper condition and so combined at the time these graphs were made as to produce large fluctuations.

Temperatures were recorded from a standardized maximum-minimum thermometer kept in a standard shelter near the auxograph, a sling psychrometer was used to determine relative humidities, and a Livingston atmometer was used to measure evaporation. The times at which the humidity readings were taken ranged from 1:30 to 2:15 P.M.

By comparing temperature, humidity, and atmometer data with the graphs it was found that climatic conditions affect the magnitude of diurnal fluctuation in fruit diameters. It was also shown very strikingly that the fluctuations, *i.e.*, the amount of water withdrawn from the lemon by leaf evaporation, may be influenced by the amount of available soil moisture. For example, on July 21 and 22 (21 and 22 days after irrigation) the maximum temperature on each day was 102° F., the relative humidities were 19 and 24, the atmometer evaporations 59 and 48 cc., and the diurnal auxographic fluctuations 43 and 48 mm. respectively; while on July 28 and 29 (28 and 29 days after irrigation) the maximum temperatures were 103 and 100°, the relative humidities 26, the atmometer evaporations 56 and 48 cc., and the diurnal auxographic fluctuations 68 and 74 mm. respectively. Soil-moisture determinations showed that water penetration preceding this period had been poor.

*Record 30 a.* Text figure 7 shows a graph made when the conditions (February 4-11) were the antithesis of those represented by the graphs in figures 4, 5, 6, and 8. The temperatures were lower, the days shorter, the relative humidities much greater, and the soil contained much more available moisture. It is of interest, however, that there actually was a measur-

able fluctuation under these conditions. There being even a slight shrinkage of the fruit caused by water-withdrawal under comparatively favorable conditions, how much more severe must be the loss of water during the much more unfavorable conditions which exist during the summer?

*Record 48 d.* The effects of climatic conditions are also shown in text figure 9. In this case the increase in the amount of water drawn from the lemon and the consequent rise of the recording pen were not occasioned by high temperature, since, as may be seen in the figure, the temperature was ten degrees lower on Sunday when the shrinkage was greatest. The increase was caused by a strong wind accompanied by low humidity. A comparison of the wind velocities for these two days will help to explain not only the increase in maximum auxographic fluctuation, but also why the lemon may begin to shrink before there is a rise in temperature. During the time from 12 P.M. to 6 P.M., November 22, the maximum wind velocity was 17 miles per hour, with a total mileage of 79, while during the time from 6 P.M., November 22, until 12 P.M., November 23, the maximum velocity was 30 miles per hour, with a total mileage of 468. This lemon began to shrink both on Saturday and on Sunday mornings before there was a rise in temperature.

*Records 42 a to d.* Another example of the marked effect of excessive leaf evaporation in producing not only a daily but a much more extended period of water deficit in the fruit is shown by the auxographic record of a lemon from September 22 to October 20, 1924. At the beginning of the test this lemon was still green but almost mature and measured 5.47 cm. in diameter. From September 22 to 25 the lemon increased in size daily, being 0.5 mm. larger on the 25th than on the 22d. On September 26 a dry wind set in and continued for three days. In the early morning of September 29 (the time of day when the lemon shows its greatest turgidity) this fruit was 0.7 mm. smaller in diameter than it was on the morning of September 26. It is of interest to note that, although other climatic conditions were more favorable than during the four days previous to the dry wind, during which time the lemon gained 0.5 mm. in diameter, it was five days after the wind ceased before this lemon regained its previous size. This means that for eight days and nights there was a marked water deficit in the lemon. At the end of this eight-day period the lemon again began to grow, and by October 19, or in sixteen days, it had increased 1.9 mm. in diameter.

*Record 10 d.* This lemon had a diameter of 5.22 cm. at the beginning of the test. The test began on Monday, November 26 and lasted until Saturday, December 1. The maximum daily temperatures were 84, 74, 77, 75, 56, and 61° F. respectively. The diurnal auxographic fluctuations for the same days were 16, 26, 18, 13, 2, and 15 mm. respectively. The greater diurnal fluctuations in the size of the lemon on Tuesday and Saturday as compared with those on the preceding days were due to a strong dry

wind which began to blow on the nights preceding these days and continued to blow until the following nights. That high temperature was not the cause of these excessive withdrawals of water from the lemon may be seen by comparing the temperatures of Tuesday and Saturday with those of the preceding days. The slightness of fluctuation on Friday was occasioned by continued cloudiness with a light shower at noon and another in the late afternoon. The effect of the dry wind on Tuesday in producing a persistent water deficit is again shown in this record, since the lemon did not again, even at night, regain its previous size until Friday, when the weather was cloudy and rainy. The dry wind on Saturday had a similar effect.

*Record 32 e.* How quickly the water content of the lemon fruit responds to changes in climatic conditions affecting leaf evaporation is indicated by a portion of record 32 *e*, shown in text figure 10. The day (August 11) began clear and the pen began to rise about 7 A.M. It continued to rise, as the lemon contracted, until about 11 A.M., when the sky became clouded and a light shower fell. This was followed by almost complete sunshine until 2 P.M., when another light shower fell. Following this there were sunshine and clouds intermittently until about 4 P.M., after which time the sun shone until evening. The graph (figure 10) shows that the times of changes in contraction and expansion of the lemon corresponded very closely with the times of climatic changes just mentioned. The wetting of the leaves caused a temporary cessation of evaporation, and the lemon not only ceased to contract but actually began to expand. At about 4:30 P.M., the usual time at which the amount of evaporation from the leaves became less than the amount of water supplied by the roots, the lemon began to expand.

*Records 34 d, 51 c, 53 a, and 66 c.* To secure further evidence on the correlation between climatic conditions and the contraction or expansion of the lemon, an attempt was made to regulate at least partially these conditions. In this case an auxograph was attached to a lemon, and then the foliage of the tree bearing it was sprayed with water, every two or three minutes, for an hour. As a result of the spraying the lemon began to expand, and a graph configuration similar to *a-b*, text figure 10, and to *B*, text figure 11, was made. The test was repeated with other lemons on different trees with the same general results.

It was found that climatic conditions had a marked effect upon the amount of expansion of the lemon while the tree was being sprayed. With very little air movement, comparatively low temperature, and relatively high humidity, the expansion was marked. If the climatic conditions were just the reverse, the expansion was relatively small and the pen traveled more nearly horizontally or in one case slightly upward, instead of descending.

That reduction of temperature as a result of the spraying did not have a direct effect upon the lemon is shown by the fact that a thermograph

placed beside one of the auxographs in one of the shelters showed a rise of one degree during the course of the test. It is interesting to note also that the contraction or expansion of the lemon was not governed entirely by the leaves attached to the branch which bore it or by other adjacent leaves. This is borne out by the fact that in the different tests the branches within the shelters bore from 75 to 225 leaves which remained dry while the tree was being sprayed.

*Records 44 b and 45 b.* Auxographs were attached to lemons, one on the easterly and the other on the westerly side of the tree. Both lemons were of the same size. The easterly half of the tree was sprayed with water for one hour and ten minutes, 2:05 to 3:15 P.M. At 2 P.M. the temperature was 82° F. and the relative humidity 41; at 3:20 P.M. they were respectively 80° and 36. From 2 to 4 P.M. the wind blew from the west at an average velocity of  $8\frac{1}{2}$  miles per hour. Text figure 11 illustrates the results of this test; graph *A* was made by the lemon attached to the westerly (unsprayed) half, and graph *B* by a lemon attached to the easterly (sprayed) half of the tree.

In dealing with an intricate problem of this nature it is realized that conclusions must not be drawn hastily, but the results of this test at least appear to give added evidence to Auchter's (1) suggestion that there may be a cross transfer of water in a woody plant. Although the westerly half of the tree was not sprayed, the lemon attached on that side ceased to contract during the spraying period on the easterly half. This fact appears to indicate either that the amount of evaporation from the westerly half of the tree was reduced, or that the lemon had access to a sufficiently increased supply of water to keep it from contracting. Taking into consideration (a) that the test was made in the open, (b) that the air temperature in the proximity of the tree remained almost constant during the test, (c) that the relative humidity next to the foliage on the westerly side of the tree was 5% lower five minutes after the spraying than five minutes before it began, and (d) that the velocity ( $8\frac{1}{2}$  miles per hour) and direction of the wind were such as to permit of only a minimum, if any, rise in humidity surrounding or within the foliage of the westerly (unsprayed) half of the tree, it does not seem that the spraying could have caused any reduction in the rate of evaporation from the westerly half of the tree.

In this connection it may be interesting to mention that the usual custom in southern California is to irrigate on only two sides of the tree row. It is often the case that the nearest irrigation furrows are from 2 to 6 feet from the tree row, thus leaving spaces 4 to 12 feet wide in the tree rows that receive no irrigation water, except by a limited amount of lateral penetration. In the latter part of the summer and in the fall, before the rains come, the water content of the soil within these areas may be at or below the hygroscopic point. The root system of the tree extends into these areas, and in spite of the great water deficit the foliage on the adjacent



sides of the tree appears to be as luxuriant as on those sides which border on the irrigation furrows. Investigation has shown that a portion of the roots pass from the dry over into the more moist areas, but the absorbing portions of many of the roots are confined entirely within the region of very limited or unavailable water supply. It would appear that such evidence gives further support to the suggestion that there may be a cross transfer of water in woody plants.

*Records 13 d and 14 d.* Comparisons of amounts of diurnal contraction and expansion were made with a lemon attached to a tree in the open and one attached to a tree covered by a cheese-cloth tent. Both lemons were of the same size. Examination of the records showed that the average diurnal fluctuation of the lemon on the tree within the tent was 54.8% of that of the one attached to the tree in the open. An atmometer was placed near each auxograph. The one placed near the auxograph within the tent evaporated 58.6% as much as the one outside. A comparison of the records for individual days showed that on those days when the wind was strong the amounts of contraction of the lemons and the evaporation from the atmometers inside and outside the tent were widely divergent, while under the reverse conditions the amounts were more nearly equal. For example, on one day there was a strong dry wind with a clear sky and the lemon within the tent contracted only 38.4% as much as the one outside, while on another day, when there was comparatively little wind and the entire day was cloudy with a light precipitation in the afternoon, the maximum contraction of the lemon within the tent was 81.4% of that of the one in the open. This appears to indicate that the amount of contraction of the lemon during the day may serve as a fairly good index of the evaporative power of the air.

Further observations concerning these and other auxographic records may be cited as follows:

1. On mornings following a dew the records show that the leaves did not begin to draw water from the fruits until most of the moisture had evaporated from the leaves and surrounding vegetation. The word "most" instead of "all" was used in the preceding sentence because it has been noted that, because the lemons are so sensitive to the effect of leaf evaporation, some of them may begin to contract while there is at least a small amount of free water on the leaves in the interior of the tree or on the vegetation under it.

Fog produced much the same effect as dew. The auxograph records showed no contraction of the lemons until the fog had cleared away, unless the fog was high and persisted well on toward noon, in which case a small amount of contraction was noted.

2. During the long, dry summer days, provided fog, dew, or clouds were not affecting factors, the lemons began to decrease in size about 6 A.M. and continued to decrease until 5 to 6 P.M. (see text figure 6). During

the winter months, under otherwise comparable conditions, the decrease in size began about 9 A.M. and continued until about 4 to 5 P.M. (see text figure 7).

3. It has also been found by observations in many groves that continued withdrawal of water from the fruits by excessive leaf evaporation may cause the fruits to fall. While this is perhaps more noticeable when the fruits are young, yet fruits of any age may be affected.

4. Several auxographic records made by lemons during periods of from one to four days of comparatively high temperature, high wind velocity, and low relative humidity show (a) that the lemons do not regain their total previous turgidity at night, and (b) that, apparently because of the relatively higher concentration of solids in these lemons when contraction begins in the morning, they do not reach a maximum of contraction commensurate with the expectation under such extenuating climatic conditions. It appears that a point has been reached beyond which, other factors being equal, the concentration within the fruits prevents the further withdrawal of water from them. This is indicated by the fact that under such conditions the curve does not have the usual more or less abrupt peak but may remain almost flat for from two to four hours.

5. The auxographic records show that the lemons may begin to contract in the morning when the temperature is not more than 55° F. and the relative humidity is 50% or more, while toward evening they may begin to expand when the temperature may be as high as 90° and the relative humidity as low as 25%. It would appear that leaf evaporation must be cuticular rather than stomatal, which is in accord with the statement of Coit and Hodgson (5) that by the time the leaf becomes mature the guard cells of the stomata have very largely ceased to function, remaining in a closed or nearly closed condition, and that 40 to 50% of the leaf evaporation takes place through the upper epidermis. May the explanation be that in the morning the ratio of total solids to water content is lower than it is toward evening? The records discussed in the preceding paragraph would indicate that this factor might have a bearing. Toward the late afternoon, though the temperature be higher and the relative humidity lower than in the morning, a certain equilibrium in the sap-concentration may have been reached which would prevent the leaves from drawing water from the fruits faster than it could be supplied under the prevailing climatic conditions.

#### CONCLUSIONS

The lemon fruit has proved to be well suited to a study of water deficit produced by excessive leaf evaporation, because of its size, water content, and semi-flexibility of structure, and because the leaves lack the ability conservatively to regulate evaporation. While tests have not been made, it seems probable that other species of *Citrus* will prove adaptable to similar studies.

The records of the auxograph have shown that the lemon fruit is very sensitive to changes in water content of the leaves, as affected by the amount of moisture in the soil and by climatic conditions. The leaves themselves may not wilt until the wilting coefficient of the soil has been reached, but the fruits may begin to suffer long before. For this reason it would seem that the amount of moisture in the soil should be kept not only above the wilting coefficient but at the highest permissible maximum without injury to the root system, especially during the summer months.

That the amount of water withdrawn from the lemon is dependent, to a certain extent, upon the amount of moisture in the soil is shown by the fact that the drier the soil becomes the greater the amount of water withdrawn from the lemon and the greater the length of its period of water deficit.

While these tests have shown that the amount of water available for the fruits is influenced by the amount of available moisture in the soil, yet they have also forced the conclusion that, regardless of the moisture in the soil, the root system of a lemon tree, when grown under arid or semi-arid conditions, is not able fully to supply the water demands under conditions producing rapid evaporation.

The records show that during periods of excessive evaporation there may be not only a daily water deficit but one which may last, during the night as well as during the day, for at least three or four weeks at a time. That such a deficit must have a profound effect upon the fruit would appear to be evident. It must materially affect the size, texture, amount and nature of solids, flavor, keeping quality, etc., of the fruit.

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# HASTENING THE SPROUTING OF DORMANT POTATO TUBERS

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## INTRODUCTION

Freshly harvested potato tubers, if replanted under conditions favorable for growth, do not sprout at once, but require a period of after-ripening. The depth of dormancy varies with the variety but is generally two months or more.

This delay in sprouting is a serious disadvantage to potato-growers. Thus, Bliss Triumph seed tubers grown in northern states, such as Maine or Nebraska, do not go through their rest period in time for planting in Bermuda, Florida, Cuba, or other southern localities in which an early crop is produced for the winter market. It has been necessary in such cases to obtain seed potatoes from Long Island or regions farther south, where the first crop of potatoes matures early enough to give a period of after-ripening sufficiently long to insure prompt germination in the autumn of that year. But such potatoes have been found generally to be subject to infection by degeneration diseases which either greatly reduce the yield of crop or cause practically a complete failure. It would be of great value to potato-growers if a method of shortening the rest period could be devised so that disease-free northern-grown seed potatoes could be taken at once after digging to southern localities and used as seed for the early winter crop.

Again, in many southern states a second crop of potatoes is grown in the same year on the same piece of land; but the sprouts are slow in starting and the stand obtained is irregular, in consequence in large measure of the dormancy of a certain proportion of the buds planted. Hence, the yield per acre is decreased materially below the normal productive capacity of the land.

Experiments for the purpose of finding a method of chemical treatment that would shorten this rest period and permit early sprouting were undertaken. While the work at present is incomplete, certain of the treatments have given hopeful results, and this paper is a preliminary report of the progress made.

## METHODS AND VARIETIES

In some experiments the tubers, both whole and cut into suitable sizes for planting, were soaked in aqueous solutions of different chemicals of

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

gradually increasing concentrations for varying lengths of time, and in others they were exposed in closed containers to gases or vapors from numerous volatile compounds. After treatment, the potatoes were planted in sawdust or in soil, in the dark or in a greenhouse during cold weather, or in the Institute gardens during the summer months.

The varieties used were: in December, 1924, Irish Cobbler, Rural New Yorker, and Green Mountain from Maryland and New Jersey; in January, 1925, McCormick from Washington, D. C., and New Jersey, Green Mountain from New Jersey; in February, 1925, Bliss Triumph from Bermuda; in April, 1925, Spaulding's Rose from Florida. Since the potatoes mentioned above were not deeply dormant, the results of the experiments with them were used merely to indicate the treatments that gave the most promise of success. The conclusions reported in this paper are based for the most part on the experiments with potatoes from the following sources: Irish Cobbler from Long Island, 1925 crop; Irish Cobbler, Bliss Triumph, and Early Ohio, 1925 crop, grown in Institute gardens, treated and replanted within one month from the time of digging.

Early forcing of sprouts was obtained with all these varieties by one or more of the chemicals used except in the case of Early Ohio. In the only experimental series with tubers of this variety, none of the treatments used was successful.

## RESULTS

### General Statement

Two hundred twenty-four different chemicals were tested and about 3,000 separate experimental lots were used in selecting suitable concentrations and periods of treatment. In this report only those that gave the best promise of success will be discussed, but it is hoped that in a later publication a complete list of the chemicals used may be given, together with the concentrations and lengths of treatments.

Ethylene chlorhydrin and the thiocyanates of sodium and potassium stood out prominently in these tests, giving the best and most consistent results in forcing the germination of dormant tubers.

Dichloroethylene, trichloroethylene, xylol, and carbon bisulfid also gave good, but somewhat less favorable, results. Experimental work with them is being continued.

Ethyl nitrite, furoic acid, methylene chlorid, furfural, bleaching powder, bromoform, and tribromphenol gave favorable results in some cases but not in others. The cause of this irregularity is not known at present.

Thiourea represents a special case. Solutions of it in proper concentration consistently caused the development of more than one bud per eye, sometimes as many as eight, and in addition caused the sprouting of more than one eye on each seed-piece.

So far as I know, this is the first report regarding the effect of the above-

mentioned substances upon the earliness of sprouting of potato tubers. The use of the following chemicals for this purpose was suggested in 1909 by McCallum (1): ethyl bromid, ethylene dichlorid, carbon tetrachlorid, ammonia, bromine, and gasoline. His experiments with these substances were repeated and favorable results were obtained with the first three, especially with ethylene dichlorid and ethyl bromid. Rosa (2) recommended the use of solutions of sodium nitrate. These were tried in many experiments, and under certain conditions good results were obtained.

#### Results with Ethylene Chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ )

Of all the chemicals tried, ethylene chlorhydrin gave the greatest promise of successful practical application for breaking the dormancy of potatoes. Since it is a volatile substance that mixes with water in all proportions, treatments were made either by soaking in solution or by exposure to vapors.

With Bliss Triumph, soaking cut tubers for one hour in solutions varying in concentration from 10 cc.<sup>2</sup> per liter to 3 cc. per liter hastened sprouting (Pl. X, fig. 1). Freshly dug Irish Cobblers required about 4 cc. per liter for two hours.

The volatility of ethylene chlorhydrin permitted its application as a vapor by three different methods: first, by placing on top of the tubers in closed containers shallow pans from which the ethylene chlorhydrin evaporated. In this case, cut tubers required about 16 hours' treatment with an amount of ethylene chlorhydrin equivalent to about 0.5 cc. for each liter of space in the vessel. The results shown in figure 2 were obtained with Bliss Triumph, 1925 crop, dug July 22, 1925, treated and planted August 11, 1925, photographed October 6, 1925.

It was found possible to treat the whole tubers by this method, the requirement being 0.5 to 1.0 cc. ethylene chlorhydrin per liter of space in the container for a period of twenty-four hours.<sup>3</sup> Figure 3 shows the results with Irish Cobbler, freshly harvested 1925 crop. Lot A received 10 cc. ethylene chlorhydrin in a 17.5-liter container for 24 hours. Lot B received no treatment. Second-crop young tubers developed on the vines from the treated lot before any sprouts of the untreated lot appeared above ground. The percentage germination was 65.

Experiments are being carried out to determine whether the effect of this treatment will be retained in the tuber during subsequent storage and, if so, for what storage period. The preliminary results indicate that the effect of the treatment holds over in the tuber during storage for at least three weeks.

<sup>2</sup> The ethylene chlorhydrin referred to in this paper is a commercial grade known as "40 percent ethylene chlorhydrin." The concentrations given! do not refer to the anhydrous chemical.

<sup>3</sup> Later experiments showed that when whole tubers were treated better results were obtained if the potatoes were allowed to stand in air after treatment for a day before planting.

A second method of applying the ethylene chlorhydrin consisted in placing the potatoes in containers that could be closed, arranging the tubers in layers about six inches deep, covering each layer with burlap saturated with a solution of ethylene chlorhydrin, closing the container and letting it stand over night (about 16 hours). The concentration of ethylene chlorhydrin used to saturate the burlap cloth was 100 cc. per liter. The results obtained varied from top to bottom of the container, indicating that this method gave an uneven distribution of the vapor inside the container. Further experiments may remedy this defect.

The third method consisted in dipping the potatoes into a solution of ethylene chlorhydrin, removing them at once and placing them in closed containers for about 16 to 24 hours. A uniform amount of liquid was thus distributed over the surface of the potatoes. The ethylene chlorhydrin evaporated, producing within the vessel a vapor, the concentration of which varied with the amount of chemical in solution. With cut tubers a dipping solution containing about 30 cc. of ethylene chlorhydrin per liter gave good results. Sprouts were visible at the surface of the tuber seven days after treatment, and, when planted two inches deep in flats indoors, the plants appeared above ground in another week. In the one series of experiments carried out in this way, uniform results were obtained at the top and bottom of the container. However, further work will be necessary to determine the relation of the size and shape of the container to the problem of obtaining a uniform distribution of vapor.

#### Results with Sodium and Potassium Thiocyanate (NaCNS and KCNS)

Sodium and potassium thiocyanate solutions gave notably good results, although the concentration margin between stimulation and injury was not large. With cut tubers in deeply dormant condition, soaking for one hour in 3-percent or 2-percent solutions forced early sprouting of Irish Cobbler and Bliss Triumph, but 1 percent was noticeably less effective and 4 percent caused loss by rotting. For tubers in the latter part of the rest period, 3 percent for one hour was found to be too strong, but 1 percent for one hour was favorable, as was also 0.25 percent for two hours. The results with KCNS are shown in figure 4, Plate XI; lot *B* shown in this figure was treated with 2 percent KCNS for one hour, lot *A* with  $H_2O$  for one hour, and lot *C* with 0.5  $M$   $NaNO_3$  for one hour. The variety used was Irish Cobbler, Long Island 1925 crop, treated and planted July 21, 1925, photographed September 20, 1925.

#### Results with Thiourea ( $NH_2CSNH_2$ )

Solutions of thiourea caused the development of more than one bud per eye, and more than one eye per seed-piece. Figure 5 shows the results obtained by soaking cut potatoes in 1-percent thiourea for one hour. In other experiments, not illustrated in this paper, as many as eight buds



developed from one eye. The uniformity with which this result was produced is shown by the fact that, out of 20 pieces treated, 18 formed two or more buds at each eye. The concentration necessary to cause this multiple-bud development varied from about 4 percent for dormant buds to about 1 percent for buds just beginning to sprout.

Solutions of thiourea, therefore, overcame the inhibition which the main bud exercises over the subsidiary buds at each eye and also partially nullified the capacity of the apical bud to prevent the growth of basal buds. It is planned to give further details on this point in a paper that is to follow.

#### Results with Ethylene ( $C_2H_4$ )

Ethylene was used in concentrations varying from 75 percent to one part in five millions for periods varying from one hour to seven days. The results were not encouraging, and in no case did the stimulation produced compare favorably with that caused by some of the other chemicals used. Rosa (3) reported that when the ethylene treatment was continued for a longer time, in his experiment for one month, the dormant period of the tubers was markedly shortened.

#### Results with Sodium Nitrate ( $NaNO_3$ )

With potatoes that were not very dormant, 0.5  $M$   $NaNO_3$  solution for one hour generally gave good forcing action, but the range of favorable concentration was small, 0.3  $M$  solution being ineffective in all cases. Some injury was caused by 0.5  $M$  solution for one hour when used with potatoes late in the dormant period. The sprouting of recently dug Bliss Triumph was forced by one hour's treatment with 0.6  $M$ , but 0.4  $M$  gave results that were little better than those of the check lot. Freshly harvested Irish Cobblers did not respond to treatment for one hour in 0.7  $M$ , 0.5  $M$ , or 0.3  $M$  sodium nitrate.

#### Results with Other Chemicals

Favorable results were obtained with the following chemicals, using the given concentrations for 16 hours with cut tubers, and for 24 hours with whole tubers:

Ethyl bromid, 2 cc. in a 17.5-liter space with cut tubers, and 3 cc. in a 17.5-liter space with whole tubers; ethylene dichlorid, 1 cc. in a 17.5-liter space with cut tubers, and 2 cc. in a 17.5-liter space with whole tubers (the results with ethylene dichlorid are shown in figure 6); carbon bisulfid, 1 cc. in 35-liter space with cut tubers, and 2 cc. in 35-liter space with whole tubers; dichloroethylene, 3 cc. in 17.5-liter space for cut tubers, 8 cc. in 17.5-liter space for whole tubers; trichloroethylene, 2 cc. in 35-liter space for cut tubers, 5 cc. in 35-liter space for whole tubers.

### Concentration of Chemicals as Related to Stage of Dormancy

It was found that concentrations favorable for treating freshly harvested tubers caused injury to tubers that were out of the dormant period and ready for immediate sprouting. The concentrations referred to in this paper are those that were found satisfactory for potatoes treated within about one month after digging. The concentrations given will serve to guide experimenters who wish to make tests with any of these chemicals. In such experiments it will be well to try concentrations stronger and weaker than those mentioned.

### COST OF TREATMENTS

If a practical method could be devised for using these chemicals for hastening the germination of dormant potatoes, their cost would not be prohibitive. Thus, ethylene chlorhydrin (40 percent commercial grade such as used in these experiments) costs 30 cents per pound. If it were used at the concentrations found favorable for forcing potatoes, it is estimated that the cost of the treatment would be approximately 4 cents per bushel; the estimate for trichloroethylene is 1 cent per bushel, and for ethylene dichlorid  $\frac{1}{3}$  cent per bushel.

### SUMMARY

The experiments were undertaken for the purpose of finding a method of hastening the germination of dormant potato tubers.

Two hundred twenty-four different chemicals were tested, and about 3,000 separate experimental lots were used in selecting suitable concentrations and periods of treatment.

The methods used consisted either in soaking the tubers (both cut and whole), usually for one hour, in aqueous solutions of different chemicals of gradually increasing concentrations, or in exposing them for varying lengths of time in closed containers to the vapors from numerous volatile compounds.

It was found that the vapors of ethylene chlorhydrin ( $\text{ClCH}_2\text{CH}_2\text{OH}$ ) were remarkably effective in causing prompt germination of dormant potatoes. With Irish Cobbler, 1925 Long Island crop, the treated lot produced vines two feet high, bearing second-crop tubers 1 cm. in diameter before the checks appeared above ground. Further work is being done to determine whether a standardized procedure for applying the ethylene chlorhydrin treatment can be worked out. There can be no doubt of its effectiveness. Only its practicability is now in question.

Sodium and potassium thiocyanate solutions gave excellent results in forcing early sprouting. Irish Cobbler, Long Island 1925 crop, treated and planted July 21, 1925, gave 100 percent germination. Sprouts appeared above ground one month after planting and in two months produced plants 24 inches high on which young tubers were beginning to form. The un-

treated tubers at this time had not yet appeared above ground. There is some doubt at present whether there is sufficient range of concentration between injury and effectiveness in order that a successful commercial method can be developed. Further experiments on this point are being made.

Other chemicals that gave favorable results were: dichloroethylene, trichloroethylene, carbon bisulfid, ethylene dichlorid, xylol, and ethyl bromid. Tests with these are being continued.

Thiourea represented a special case. Solutions of it in proper concentration not only forced prompt sprouting, but consistently caused the development of more than one sprout per eye, sometimes as many as eight, and in addition induced the development of more than one eye on each potato. It thus overcame the inhibiting effect which the main bud in each eye exerts upon the subsidiary buds, and also partially nullified the capacity of the terminal bud to prevent the development of basal buds on the same seed-piece.

The results with ethylene were unsatisfactory. In no case tried were its stimulating effects comparable with those of the substances principally referred to in this paper. The periods of exposure to ethylene, however, were for a few hours or days only, and not for the long period (one month) by means of which Rosa (3) obtained favorable results with ethylene.

I wish to express my thanks to Dr. William Stuart of the United States Department of Agriculture for furnishing generous quantities of experimental seed potatoes.

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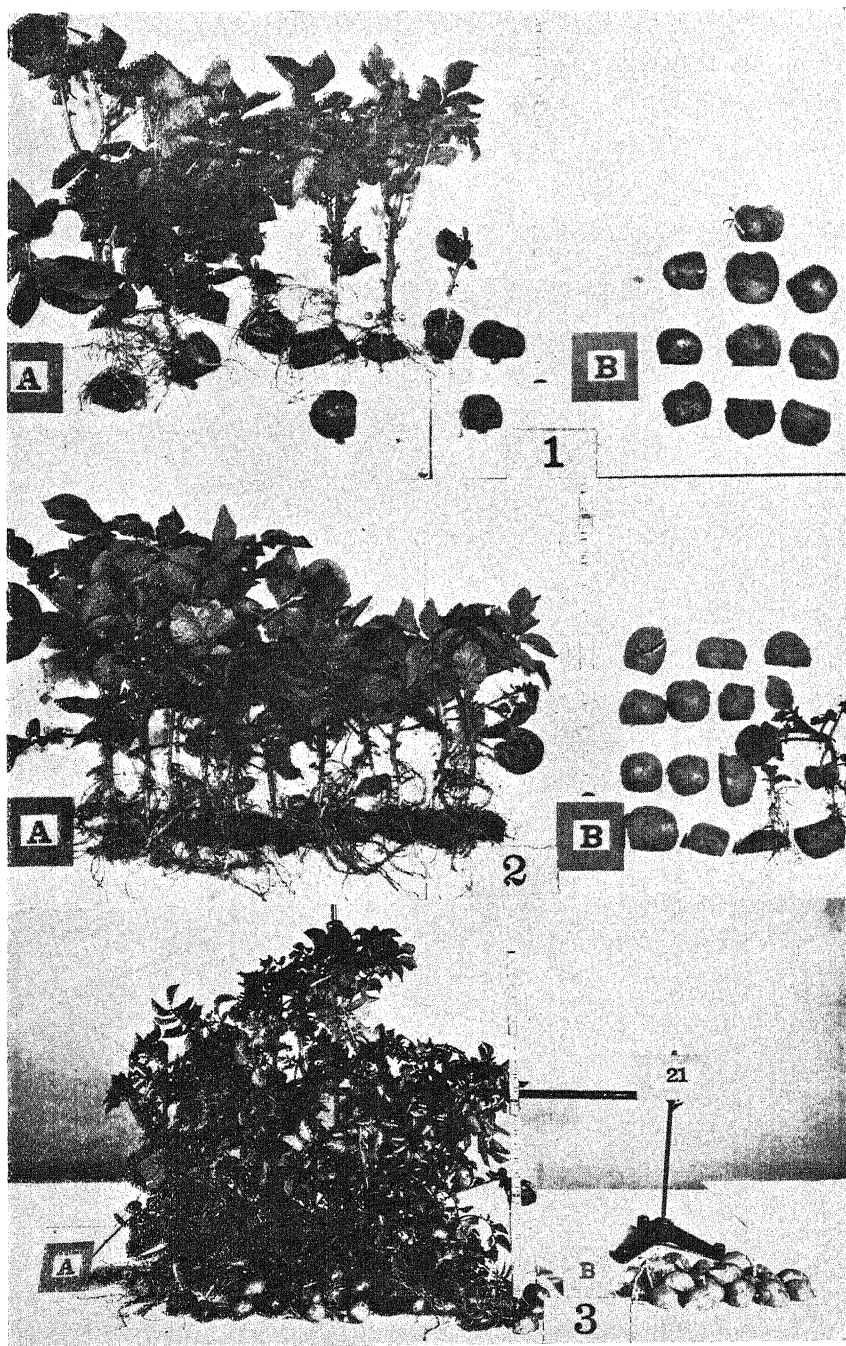
#### DESCRIPTION OF FIGURES

##### PLATE X

FIG. 1. *A*, cut tubers soaked 1 hour in a solution of 10 cc. ethylene chlorhydrin in 1 liter; *B*, soaked 1 hour in water; Bliss Triumph, 1925 crop; dug July 22, treated and planted August 11, photographed October 6.

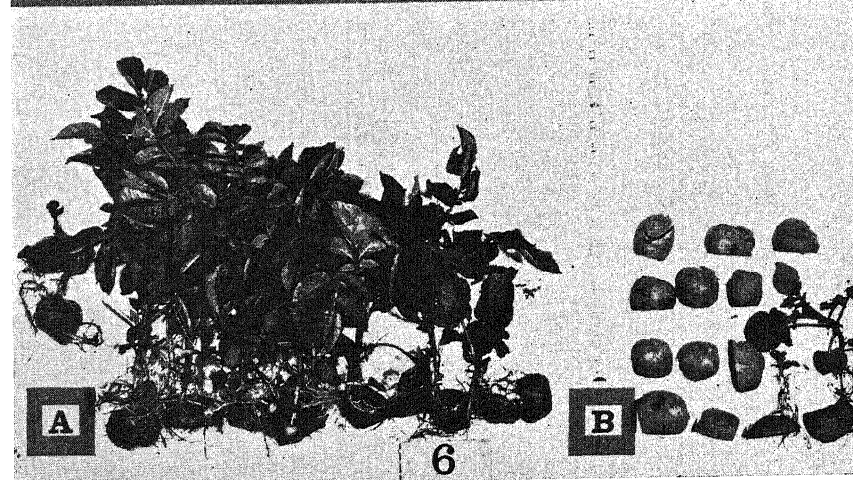
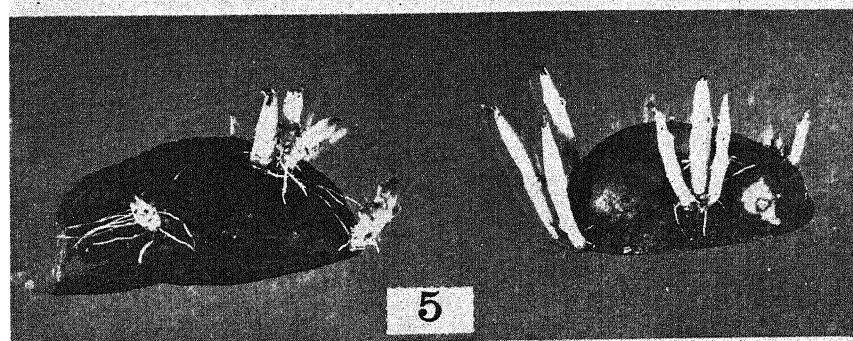
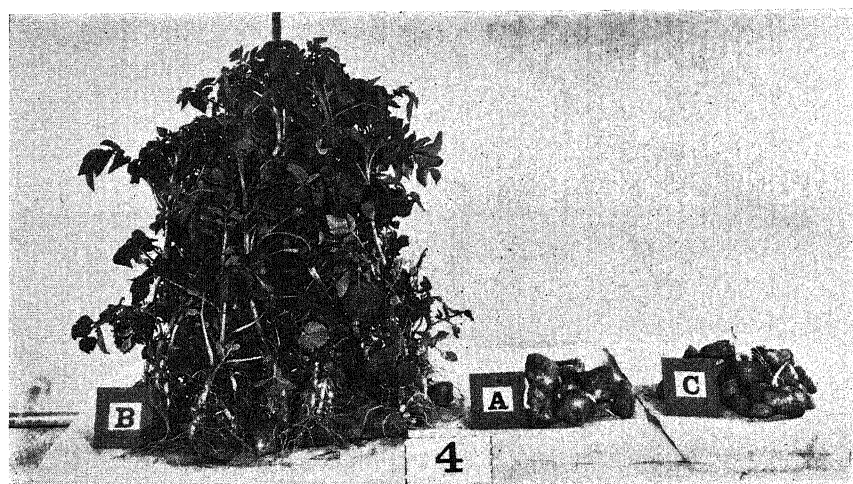
FIG. 2. *A*, ethylene chlorhydrin, 1 cc. in 2-liter space; cut tubers treated 16 hours; *B*, check, not treated; Bliss Triumph, 1925 crop; dug July 22, treated and planted August 11 and 12, photographed October 6.

FIG. 3. *A*, ethylene chlorhydrin, 10 cc. in 17.5-liter space; whole tubers treated 24 hours; *B*, check, not treated; Irish Cobbler, Long Island 1925 crop; treated and planted July 22 and 23, photographed October 6.



DENNY: SPROUTING OF DORMANT TUBERS





DENNY: SPROUTING OF DORMANT TUBERS



## PLATE XI

FIG. 4. *B*, cut tubers soaked 1 hour in 2-percent solution of potassium thiocyanate; *C*, soaked 1 hour in 0.5 *M* solution of sodium nitrate; *A*, check, soaked 1 hour in water; Irish Cobbler, Long Island 1925 crop; treated and planted July 21, photographed September 20.

FIG. 5. Effect of thiourea in causing the development of more than one bud per eye. and of more than one eye per seed-piece; Rural New Yorker, not dormant, soaked 1 hour in 1 percent thiourea solution, both tubers treated.

FIG. 6. *A*, ethylene dichlorid, 0.5 cc. in 17.5-liter space; cut potatoes treated 16 hours; *B*, check, not treated; Bliss Triumph, 1925 crop; dug July 22, treated and planted in field August 11 and 12, photographed October 6.



# GERMINATION OF SEEDS UNDER WATER

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(Received for publication December 4, 1925)<sup>1</sup>

## INTRODUCTION

It is a well known fact that seeds of many common crops are not only unable to germinate in water, but gradually lose their viability and finally come to death in it (2, 12, 17). According to Shull (15), however, some kinds of seeds can retain their viability for several years under water. Crocker (4) believed that "the discrepancy between these results and those of Mazé is probably best explained by the fact that Shull was working with the seeds of wild plants, while Mazé was dealing with cultivated species."

Kraus (9) studied the germination of *Triticum vulgare*, *Secale cereale*, *Avena sativa*, *Hordeum vulgare*, *Zea Mays*, *Sinapis alba*, *Polygonum Fagopyrum*, *Pisum sativum*, *Cucurbita Pepo*, *Lupinus luteus*, *Vicia Faba*, *Helianthus annuus*, *Brassica rapa*, *Lepidium sativum*, *Vicia sativa*, *Trifolium pratense*, *Hyacinthus candicans*, and *Lathyrus grandiflorus* in tap water. The last seven species germinated in water and also made considerable later growth. *Cucurbita Pepo* and *Lupinus luteus* showed no germination; all others pushed hypocotyls out of testas without any further growth. He also found that aëration of the water or the addition of small amounts of  $H_2O_2$  was helpful for the growth of germinating seeds.

Kinzel (8) and Lehman (10), in their work on the effect of light on germination, and Godlewski (6), in studying anaërobic growth, observed germination of seeds under water. Kinzel worked on many wild-land and marsh-plant seeds. He obtained especially good germination in water with *Hypericum*, *Rhododendron*, *Azalea*, *Calluna*, *Primula*, *Verbascum*, *Mimulus*, *Digitalis*, *Veronica*, *Campanula*, etc.

In the first part of this work the writer reports the results of a comparison of germination in water and on filter paper. Many different species were included in the study in order to determine whether there is any phylogenetic relation in ability to germinate under water. In the latter part, attention is given to the effect of temperatures on the germination under water.

Nearly all the seeds for the experiment were bought from the Vaughan Seed Company and Stumpp and Walter Seed Company in New York, and were of the crop of 1923.

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

## EXPERIMENTAL RESULTS

## Comparison of Germination under Water and on Wet Filter Paper

For germination under water, seeds were kept at the bottom of 100 cc. Erlenmeyer flasks filled with distilled water. As checks, the same number of seeds were put on moistened filter paper in Petri dishes. The flasks were put side by side, and the dishes were placed in piles in a greenhouse. The temperatures in the greenhouse varied from 15° to 33° C. The water in the flasks was renewed once in two weeks. The germination record was taken once a week, and seeds in the water were counted as germinated only when hypocotyls or sometimes plumules (epicotyls) also started healthy growth. The experiment was continued for 42 days. The results are summarized in tables 1A-1C.

TABLE 1A. *Seeds Giving no Germination under Water*

Names	No. Seeds per Cul- ture	Percent Germi- nation in Petri Dish	Percent Germi- nation under Water
<i>Festuca pratensis</i> (meadow fescue) . . . . .	100	96.5*	0
<i>Festuca elatior</i> (fall fescue) . . . . .	100	86.5	0
<i>Lolium perenne</i> (English rye grass) . . . . .	100	52.0	0
<i>Andropogon sorghum</i> (common sorghum) . . . . .	25	82.0	0
<i>Avena sativa</i> (oats, without glumes) . . . . .	20	100.0	0
<i>Triticum vulgare</i> (Marquis wheat) . . . . .	25	98.0*	0*
<i>Zea Mays</i> (field corn) . . . . .	5	100.0	0
<i>Coix lacryma</i> (Job's tears) . . . . .	4	100.0	0 (0)†
<i>Setaria macrochaeta</i> . . . . .	50	97.0	0 (0)
<i>Raphanus sativus</i> (radish) . . . . .	25	72.0	0
<i>Petroselinum hortense</i> (parsley) . . . . .	50	77.0	0 (10)
<i>Brassica oleracea</i> (cabbage) . . . . .	50	86.0	0 (0)
<i>Iberis</i> (candytuft) . . . . .	50	82.0	0 (0)
<i>Cosmos</i> . . . . .	50	87.0	0 (0)
<i>Aster</i> . . . . .	50	65.0	0 (0)
<i>Centaurea</i> . . . . .	25	84.0	0 (0)
<i>Carthamus tinctorius</i> (saffron) . . . . .	10	60.0	0 (0)
<i>Capsicum annuum</i> var. <i>longum</i> (pepper) . . . . .	25	74.0	0 (44)
<i>Solanum melongena</i> (egg plant) . . . . .	25	92.0	0 (66)
<i>Allium Cepa</i> (onion) . . . . .	25	60.0	0 (0)
<i>Asparagus officinalis</i> . . . . .	25	58.0	0 (34)
<i>Cucumis Melo</i> (muskmelon) . . . . .	10	95.0	0 (5)
<i>Cucurbita maxima</i> (squash) . . . . .	5	80.0	0 (0)
<i>Viola tricolor</i> (pansy) . . . . .	100	72.0	0 (0)
<i>Fagopyrum esculentum</i> (buckwheat) . . . . .	20	92.5	0
<i>Salvia officinalis</i> (sage) . . . . .	50	84.0	0 (0)
<i>Hyssopus officinalis</i> (hyssop) . . . . .	100	76.5	0
<i>Berberis Thunbergii</i> . . . . .	20	72.5	0 (0)
<i>Abutilon</i> . . . . .	25	98.0	0
<i>Phlox Drummondii</i> . . . . .	25	60.0	0 (0)
<i>Pisum sativum</i> (Alaska pea) . . . . .	10	100.0	0
<i>Phaseolus</i> (bean) . . . . .	5	90.0	0
<i>Lupinus</i> . . . . .	20	50.0	0 (5)
<i>Medicago sativa</i> (alfalfa) . . . . .	100	94.5	0

\* All these numbers are the means of duplicated tests.

† The number in parentheses in the last column shows the percentage of germination of the seeds after being taken out of the water and placed on moist filter paper.

TABLE 1B. *Seeds Giving Poorer Germination under Water than on Moist Filter Paper*

Names	No. Seeds per Cul- ture	Percent Germi- nation in Petri Dish	Percent Germi- nation under Water
<i>Alopecurus pratensis</i> (meadow foxtail).....	100†	85.0*	67.0*
<i>Festuca duriuscula</i> (hard fescue).....	100	82.0	9.5
<i>Cynosurus cristatus</i> (crested dog's tail).....	100	48.5	16.5
<i>Bromus brizaeformis</i> (brome-grass).....	10†	100.0	85.0
<i>Eragrostis elegans</i> (love grass).....	100	81.5	66.5
<i>Nicotiana</i> .....	100	84.0	68.0
<i>Lycopersicum lycopersicum</i> (tomato).....	50	71.0	30.0
<i>Salpiglossis</i> .....	100	76.0	38.5
<i>Trifolium repens</i> (white clover).....	100	84.5	66.0
<i>Mimosa pudica</i> (sensitive plant).....	10	90.0	80.0
<i>Ocimum basilicum</i> (basil).....	50	68.0	51.0
<i>Thymus vulgaris</i> (thyme).....	100	63.5	25.0
<i>Satureja hortensis</i> (summer savory).....	100	59.5	5.0
<i>Marrubium vulgare</i> (horehound).....	100	44.5	2.0
<i>Origanum Majorana</i> (marjoram).....	100	87.5	62.0
<i>Dianthus</i> (carnation).....	50	99.0	89.0
<i>Allium porrum</i> (leek).....	50	72.0	24.0
<i>Alyssum</i> .....	100	91.5	63.0
<i>Ruta graveolens</i> (rue).....	50	51.0	15.0
<i>Anthemis nobilis</i> (chamomile).....	100	92.5	86.0
<i>Sesamum orientale</i> (bene).....	50	99.0	21.0
<i>Clematis virginiana</i> .....	25	96.0	58.0
<i>Campanula medium</i> (Canterbury bell).....	100	83.0	52.0
<i>Amaranthus</i> .....	100	94.0	87.0
<i>Hunnemannia</i> .....	50	93.0	1.0

\* All these numbers are the means of duplicated tests.

† Without glumes.

TABLE 1C. *Seeds Showing Good Germination under Water*

Names	No. Seeds per Cul- ture	Percent Germi- nation in Petri Dish	Percent Germi- nation under Water
<i>Phleum pratense</i> (timothy).....	100†	93.0*	90.0*
<i>Axonopus compressus</i> (carpet grass).....	100	89.5	93.5
<i>Agrostis nebulosa</i> .....	100†	91.5	96.0
<i>Poa compressa</i> (Canadian blue grass).....	100†	84.5	94.5
<i>Cynodon dactylon</i> (Bermuda grass).....	100†	79.0	95.5
<i>Lactuca sativa</i> (lettuce).....	50	95.0	95.0
<i>Artemisia Absinthium</i> (wormwood).....	100	85.0	89.0
<i>Daucus Carota</i> (carrot).....	25	68.0	66.0
<i>Apium graveolens</i> (celery).....	100	92.0	88.0
<i>Portulaca</i> .....	100	78.0	81.0
<i>Mesembryanthemum crystallinum</i> (ice plant)	100	82.0	87.0
<i>Antirrhinum</i> (snapdragon).....	100	88.0	92.0
<i>Melissa officinalis</i> .....	100	80.0	74.5
<i>Celosia</i> (cockscomb).....	100	94.5	94.5
<i>Oenothera</i> (evening primrose).....	100	85.0	84.0
<i>Silene latifolia</i> .....	100	97.5	94.5
<i>Solanum Dulcamara</i> (bittersweet).....	50	99.0	98.0
<i>Petunia</i> .....	100	93.5	91.5

\* All these numbers are the means of duplicated tests.

† Without glumes.

When, instead of 100, only 10 hulled grains of *Festuca pratensis*, *Festuca elatior*, *Lolium perenne*, and only 10 seeds of *Medicago sativa* were taken in each flask, they germinated 25.5, 0.5, 14, and 95 percent respectively, thus showing an increased germination under water when a smaller number of seeds per flask were taken.

These experiments are considered preliminary especially on account of the uncontrolled temperature and light conditions.

Some unfavorable conditions obtain in the water cultures. Thus, the great depth of water makes oxygen inaccessible (9); furthermore, distilled water was used exclusively and renewed only once in two weeks. Out of 78 genera of 24 families, 43 genera germinated in the water. Even some of the 35 genera which did not germinate under the conditions of the experiments did germinate if fewer seeds were placed in the flasks, so that the small amount of oxygen present was used by fewer seeds.

Amongst the 43 genera which germinated in the water, 18 genera did not show any decided difference between the germination in the water and that on filter paper, and 2 genera (*Cynodon dactylon* and *Poa compressa*) germinated better under water than on blotters. Egg plant, pepper, and asparagus seeds kept their vitality well during 6 weeks' submersion. The ability to germinate under water obtained more generally for small seeds, and was not related to phylogeny or to the kinds of reserve material in the seeds.

### Germination of Seeds in Boiled Distilled Water

Hartleb and Stuzer (7) and Kraus (9) maintained that seeds are unable to germinate in boiled water on account of the absence of oxygen. Florence

TABLE 2. Germination of Seeds in Boiled Water

Names	No. Seeds per Flask	Percent Germination	No. Days Tested
<i>Phleum pratense</i> (timothy).....	25	60.0*	9
<i>Cynodon dactylon</i> (Bermuda grass).....	25	86.0	9
<i>Poa compressa</i> (Canadian blue grass).....	25	18.0	11
<i>Festuca pratensis</i> (meadow fescue).....	10	25.0	11
<i>Festuca elatior</i> (fall fescue).....	10	50.0	11
<i>Lactuca sativa</i> (lettuce).....	20	12.5 (poor)	13
<i>Artemisia Absinthium</i> (wormwood).....	25	54.0	5
<i>Anthemis nobilis</i> (chamomile).....	25	76.0	5
<i>Daucus Carota</i> (carrot).....	10	45.0	15
<i>Apium graveolens</i> (celery).....	20	52.5	21
<i>Trifolium repens</i> (white clover).....	20	51.5	5
<i>Medicago sativa</i> (alfalfa).....	10	0	—
<i>Antirrhinum</i> (snapdragon).....	25	60.0	9
<i>Ocimum basilicum</i> (basil).....	10	75.0	9
<i>Celosia</i> (cockscomb).....	25	74.0	7
<i>Dianthus</i> (carnation).....	20	70.0	7
<i>Solanum Dulcamara</i> .....	20	87.5	11
<i>Sesamum orientale</i> (bene).....	10	55.0	15
<i>Alyssum</i> .....	20	67.5	7
<i>Petunia</i> .....	25	78.0	9
<i>Mesembryanthemum crystallinum</i> (ice plant)	20	45.0	7

\* All these numbers are the means of duplicated tests.

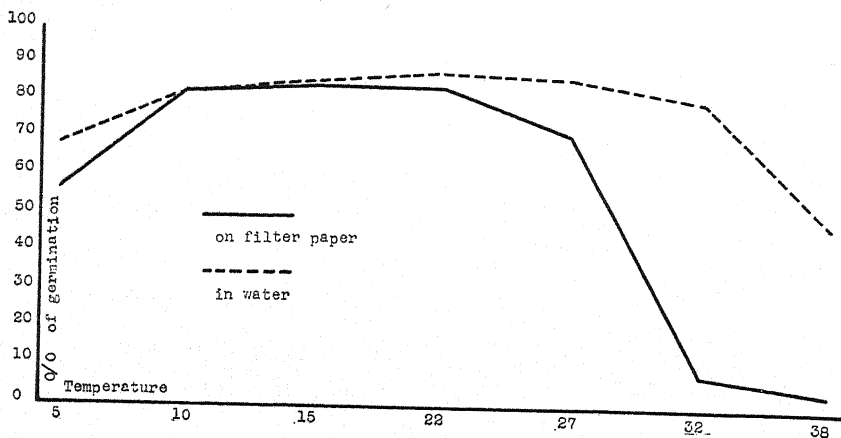
flasks of 100-cc. capacity were nearly filled with boiled distilled water and cooled quickly in ice water. As soon as the water was cool enough, seeds were put in and sealed with paraffin oil. These flasks were placed in the greenhouse as experiment *A*. When the seedlings that germinated in the flask became green, records were taken and the flask was discarded because oxygen could be liberated inside the flask by photosynthesis as soon as chlorophyll was formed. This experiment, therefore, does not show any final percentage of germination, but shows whether seeds can germinate at all in this condition. Twenty-one kinds of seeds which germinated well in water in experiment *A* were chosen for this experiment. The results obtained are shown in table 2.

These results do not agree with those of the workers above mentioned, the seeds of all species except alfalfa giving either fair or good germination. This discrepancy, however, may have been due to a difference in the method of sealing, to a difference in species, or to a more favorable alternation of temperature. Carnation, chamomile, wormwood, timothy, white clover, and *Celosia* seeds were chosen and tested for germination in Petri dishes in a sealed jar containing potassium pyrogallate for absorbing oxygen. None of these germinated.

#### Germination of Seeds in Water in Contact with Pure Oxygen

Running water (2), artificial aëration, or addition of  $H_2O_2$  (9, 12) to increase dissolved oxygen was successfully used to germinate seeds in water. In this experiment only seeds which did not germinate in experiment *A* (see table 1A) were chosen and tested for germination in the water. They were placed in contact with pure oxygen instead of with air.

Small numbers of seeds were put in 500-cc. Erlenmeyer flasks with 50 cc. distilled water, the depth of immersion being about one centimeter.



TEXT FIG. 1. Curves showing germination of white clover seeds in water and on filter paper at various temperatures; curves plotted from table 3.

In one flask of each pair the air inside was replaced with pure oxygen. Common sorghum, wheat, oats, corn, Alaska pea, onion, cabbage, muskmelon, kohlrabi, and mustard were tried. Mustard, kohlrabi, cabbage, oats, and common sorghum germinated in both conditions, and wheat, onion, and muskmelon germinated only in the oxygen flask. They all grew well and made green seedlings. Corn and pea did not germinate in either the air or the oxygen flask.

From this experiment it is clear that a number of the seeds listed in table 1A as showing no germination under water can germinate in water if the oxygen supply is increased.

### Effect of Temperature on Germination under Water

According to Akemine (1), the seeds of *Oryza sativa* germinated 94 percent at 40° C. in water, but none of them germinated on wet filter paper at the same temperature. The seeds, however, germinated as well on filter paper as in water at the optimum temperature (30°–35° C.), and again germinated better in water at the minimum (10°–12° C.).

We have observed similar results with white clover seeds, and wish to emphasize the importance of temperature effects when a comparison is made between germination in water and germination on filter paper.

To avoid other factors which affect the germination, especially in water, only 10 seeds were put with 100 cc. distilled water in 100-cc. Erlenmeyer flasks, or on filter paper in Petri dishes. The water in the flasks was not changed during the experiment. The results are shown in table 3 and in text figure 1.

TABLE 3. Germination of White Clover Seeds

Temperature	No. Seeds Tested	Percent Germination in Water			No. Seeds Treated	Percent Germination on Wet Filter Paper		
		After 2 Days	After 6 Days	After 10 Days		After 2 Days	After 6 Days	After 10 Days
5°	10 x 10	0	43	69*	10 x 10	0	13	57†
10°	10 x 10	37	83	83	10 x 10	13	83	83
15°	16 x 10	71.3	85.6	86.3	16 x 10	56.3	83.8	85.0
22°	10 x 10	82	87	88	10 x 10	69	83	84
27°	10 x 10	81	87	87	10 x 10	54	72	72
32°	16 x 10	69.4	79.4	81.3	16 x 10	6.3	8.1	8.8
38°	10 x 10	37	47	49	10 x 10	1	1	4

\* 76 percent germination after 14 days.

† 72 percent germination after 14 days.

As is shown in table 3 and in text figure 1, high temperature retards germination markedly in Petri dishes, but much less in water. At 15° C. or at lower temperatures, there was practically no difference between the water and the filter paper; but when the temperature was 32° C. or higher, nearly ten times as many seeds germinated in water as in Petri dishes. This was not merely a response of a special variety or lot of white clover

seeds, since three additional lots purchased from three different seedsmen gave the results, as shown in table 4.

TABLE 4. *Germination of White Clover Seeds*  
(10 x 10 seeds of each lot were tested for each temperature;  
the water in the flasks was not renewed)  
*Germination Test at 32° C.*

	In Water		On Wet Filter Paper	
	Percent Germination after 10 Days	Percent Hard Seeds	Percent Germination after 10 Days	Percent Hard Seeds
Lot B....	68	4	10	5
Lot C....	58	12	11	4
Lot D....	69	12	20	13

*Germination Test at 15° C.*

	In Water		On Wet Filter Paper	
	Percent Germination after 10 Days	Percent Hard Seeds	Percent Germination after 10 Days	Percent Hard Seeds
Lot B....	89	11	87	12
Lot C....	79	10	75	16
Lot D....	81	14	85	14

Several other experiments were run in Petri dishes at 32° C. to test the effect of various substrata upon the percentage of germination. Table 5 shows the results of these.

TABLE 5. *Germination of White Clover Seeds in Petri Dishes at 32° C.*

Conditions	No. Seeds Tested	Per cent Germination after 10 Days
Germination on filter paper.....	16 x 10	8.8
Germination between filter paper.....	4 x 50	8.5
Germination on filter paper in dishes, keeping NaOH container inside.....	2 x 100	8
Germination on filter paper, without cover of dish.....	2 x 100	1
Germination in quartz sand, in Petri dishes.....	2 x 100	13
Germination in river sand, in Petri dishes.....	2 x 100	39
Germination on filter paper, in Petri dishes.....	2 x 100	17.5
Germination on filter paper, with $\frac{N}{100}$ KNO <sub>3</sub> .....	2 x 100	6
Germination on potting soil, in Petri dishes.....	2 x 100	32
Germination on filter paper, first 6 hours at 5° C.....	2 x 100	5
Germination on filter paper, first 6 hours at 10° C.....	2 x 100	3.5
Germination on filter paper, first 6 hours at 15° C.....	2 x 100	3
Germination on filter paper, seeds injected with water*.....	2 x 100	5.5
Germination on filter paper, seeds sealed with wax.....	2 x 200	44
Germination on filter paper, dishes sealed with wax keeping NaOH container inside.....	2 x 200	4

\* The seeds were injected by removing and replacing the air pressure repeatedly while the seeds were under water.

None of these conditions gave germination equal to that in water.

### Effect of Seed Coat upon Germination of White Clover Seed at High Temperature

Since the rôle of seed coats in germination was worked out by Crocker (3) in 1906, many experimenters dealing with seeds giving poor germination have paid special attention to seed-coat effects.

Several experiments were made to determine whether the seed coats of white clover are a factor in the failure of germination on wet filter paper at high temperatures. Treatment of white clover seeds with concentrated sulfuric acid increases the percentage of germination on filter paper at 32° C.; 20 minutes' treatment gave a germination of 51.5 percent within 10 days on filter paper at 32° C. The portion of the seed coat where the hypocotyl breaks through was cut off with a sharp razor, and the seeds thus treated were kept at 32° C., half in water and half on filter paper. After the treatment they gave 94 percent germination in water, and 31 percent on blotters. After keeping seeds on filter paper at 32° C. for 1 day, the coats were carefully removed. The naked embryo thus obtained germinated 100 percent in water at 32° C., and 73.3 percent on blotters at the same temperature. The 26.3 percent which did not germinate on blotters germinated when they were transferred into water.

From these experiments it is seen that treatment or removal of the seed coat is effective in increasing the germination of white clover seeds on filter paper at 32° C., but better results are always obtained in water with the seeds treated in the same way.

### Effect of Varying the Partial Oxygen Pressure

Several experiments were carried out under various conditions of reduced oxygen supply. It has already been shown in table 2 that white clover seeds will germinate in the greenhouse in boiled water sealed with paraffin oil. Using 10 seeds in each Florence flask, these experiments were repeated at 32° C. and 15° C., 6 percent germinating at 32° C. and 74 percent at 15° C.

In order to learn whether the seed will germinate in total absence of oxygen as observed by Takahashi (16) with *Oryza sativa*, Crocker and Davis (5) with *Alisma Plantago*, and Godlewski (6) with *Lupinus* in sugar solution, 10 seeds were placed with 10 cc. of distilled water into a 25-cc. distilling flask. The mouth of the flask was sealed just above the side tube. The side tube was then drawn out to a capillary in one region, and connected with a high vacuum. The flask was exhausted and kept under water at 40° to 45° C. for 30 minutes, and the side tube was sealed while still under this condition. During this time the water in the flask was reduced from 10 cc. to 1 cc.; the contents of the flask were always cool because of the rapid evaporation of water. None of the seeds treated in this way germinated at 15° C., the best temperature for germination of white clover.



Germination was also tried in reduced partial oxygen pressure obtained by mixing nitrogen or hydrogen with air. One or two Petri dishes containing 100 seeds each were put on a tripod standing in a pan of water. A battery jar was inverted over the Petri dish of seeds and the tripod. This gave a water-sealed air chamber within the battery jar; 60 percent of the air inside the jar was withdrawn and replaced by the same amount of nitrogen or hydrogen.

TABLE 6. *Germination of White Clover Seeds in Hydrogen or Nitrogen Gas Mixture at 32° C.*

Conditions	Percent Germination after			
	2 Days	4 Days	6 Days	8 Days
Air.....	43*	50.5	51	51.5
60 percent N <sub>2</sub> .....	24	43	46	47
60 percent H <sub>2</sub> .....	52	67.5	69.5	70

\* These figures are the mean values of duplicated tests.

Table 6 shows that the germination of white clover was increased when the oxygen concentration of the air was reduced by mixing with it 60 percent of hydrogen. No increase in germination was obtained, however, when nitrogen was used as the diluent. The reasons for this difference between the effects of hydrogen and of nitrogen are not known.

The experiment with seeds in 60-percent H<sub>2</sub> gas mixture with air at 32° C. was repeated 9 times with 1,300 seeds under the same or slightly modified conditions. The average germination was 61.2 percent. The experiment with the control lot exposed to air at 32° C. was repeated 15 times with 2,600 seeds. The average germination was 36.1 percent. For a check on the above described experiments, germination was tested 15 times in Petri dishes with 1,500 seeds with an average germination of 8 percent.

This series of experiments was also carried out at 15° C. and at 38° C. At 15° C. the seeds germinated equally well in all conditions. At 38° C. they germinated better in H<sub>2</sub> mixture, although the percentage of germination was low. The seedlings germinated in H<sub>2</sub> gas mixture were stunted, but they were normal in N<sub>2</sub> gas mixture.

### General Considerations on Germination of White Clover Seeds

White clover seeds germinate at temperatures between 10° and 38° C., the optimum temperature being about 15° C. The demand for oxygen for the germination of the seed is very low and dissolved oxygen in water is sufficient for germination, so that the statement of Schaible (14) that seeds germinate more slowly and in fewer numbers in reduced air medium than in normal air does not seem applicable to white clover or to most of the seeds listed in table 1C. In fact, white clover seeds germinate and

grow in boiled water covered with paraffin oil. They are not, however, able to germinate in a vacuum.

This fact suggests that a slight amount of oxygen in the tissue or in the medium is necessary for germination. This was also the case for *Typha* and *Cynodon* seeds (see paper in the present issue of this JOURNAL on "The favorable effect of reduced oxygen supply upon the germination of certain seeds"), but not for *Oryza sativa*. This amount of oxygen may hardly be enough to supply energy for germination by normal respiration, but may suffice to initiate some chemical change in the seeds. Most of the white clover seeds of the lot tested were not hard. They germinated about 85 percent at 15° C. in water and on wet paper, but at 32° C. and 38° C. the germination is much poorer on filter paper than in water. This difference is much reduced when the naked embryo is used.

What is the rôle of the seed coat in retarding the germination at high temperatures on filter paper? Since enough water for germination is taken by intact seeds at lower temperatures, and since a trace of oxygen is sufficient for germination, the exclusion of oxygen or water by the coat is probably not the chief reason for the failure to germinate. Insufficient diffusion of CO<sub>2</sub> out of the seed through the coat, or simple mechanical obstruction to the increase of the volume of the embryo, also deserves consideration. This idea is not supported by experimental results at present, but may furnish a suggestion for further investigation.

Why does the naked embryo of a white clover seed germinate more poorly on filter paper than in water at high temperatures? To solve this problem, we must know the significance of the maximum temperature for germination, which sometimes is much lower than the maximum temperature for the growth of the seedling. The raising of the temperature increases the amount of respiration, and if respiration becomes so vigorous as to use up all reserve material available at the temperature, the seed will not be able to germinate (11). On the other hand, the increase of respiration caused by the raising of temperature increases the amount of toxic by-products of respiration. If these substances accumulate to a certain amount in the seeds, germination will be retarded. The amount of reserve material (18, 5) and the amount of respiration will be changed by the amount of oxygen available to the seed. The amount of toxic substances is also related to oxygen supply, and if the seed starts anaërobic respiration, these substances will become more abundant (13). If such conceptions are reasonable, we can imagine the change of maximum temperature according to the amount of oxygen in the medium for germination. Whether the increase of oxygen raises the maximum temperature or decreases it may depend not only upon the reserve materials and the construction of the seed, but also upon the character of the protoplasm itself. Without a study of the chemical changes actually going on in these germination media at high and low temperatures, speculation regarding the reason for the

better germination in water than on wet paper at high temperatures is of little value.

### Germination of Sweet Clover (*Melilotus*) Seeds

Ten previously filed seeds were placed in 100-cc. Erlenmeyer flasks with 100 cc. distilled water, or on filter paper in Petri dishes. One set out of two was kept at 15° C., and the other at 32° C. The results are given in table 7.

TABLE 7. *Germination of Sweet Clover Seeds*

Temperature	Condition	No. Seeds Tested	Percent Germination and Hard Seeds after									
			2 Days		4 Days		6 Days		8 Days		10 Days	
			Germ.	H.S.	Germ.	H.S.	Germ.	H.S.	Germ.	H.S.	Germ.	H.S.
32° C.	In water	10 x 10	30	3	73	3	88	3	88	3	89	3
	On paper	10 x 10	5	4	17	3	54	3	75	3	87	3
15° C.	In water	10 x 10	90	8	94	6	95	4	96	4	96	3
	On paper	10 x 10	98	2	98	1	99	1	99	1	99	1

In this case, there was no difference between the final percentages of germination in water and on filter paper, but at 32° C. the seeds germinated much more quickly in water than on filter paper.

### Germination of Red Clover (*Trifolium pratense*) and Alfalfa (*Medicago sativa*) Seeds

These seeds were tried without filing in water and on filter paper in the same way as other white or sweet clover seeds. The results are given in table 8.

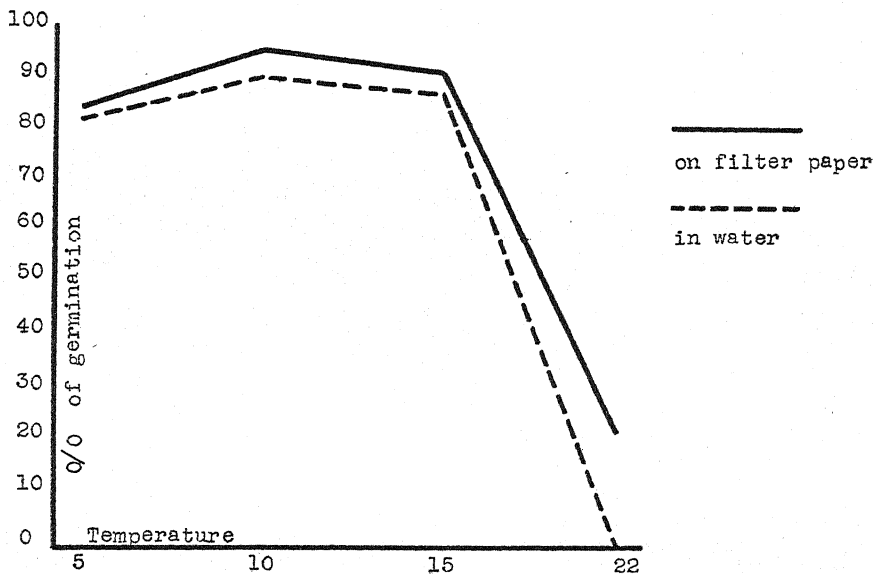
TABLE 8. *Germination of Red Clover and Alfalfa Seeds*

Kind of Seeds	Temperature	Number of Seeds Tested	Percent Germination and Hard Seeds after 10 Days			
			In Water		On Paper	
			Germ.	H.S.	Germ.	H.S.
Red clover.....	15° C.	10 x 10	88	6	87	10
	32° C.	10 x 10	68	5	72	6
	38° C.	10 x 10	3	2	58	8
Alfalfa.....	15° C.	10 x 10	90	2	93	1
	32° C.	10 x 10	49	2	82	0

From table 8 it can be seen that the results with red clover and alfalfa differed from those obtained with sweet clover in one important respect, namely, germination in water was *not better* than germination on filter paper at temperatures above the optimum.

### Germination of Celery (*Apium graveolens*) Seeds

Seeds were placed in 100-cc. Erlenmeyer flasks with 100 cc. distilled water, or on filter paper in Petri dishes. The flasks and dishes were wrapped with three sheets of black paper to avoid the favorable effect of light on germination at high temperatures,<sup>2</sup> and kept in thermostats at various temperatures. The water in the flasks was renewed once after 14 days. The results are shown in table 9 and text figure 2.



TEXT FIG. 2. Curves showing germination of celery (var. Schumacher) in water and on filter paper at various temperatures; curves plotted from table 9.

TABLE 9. *Germination of Celery Seeds*

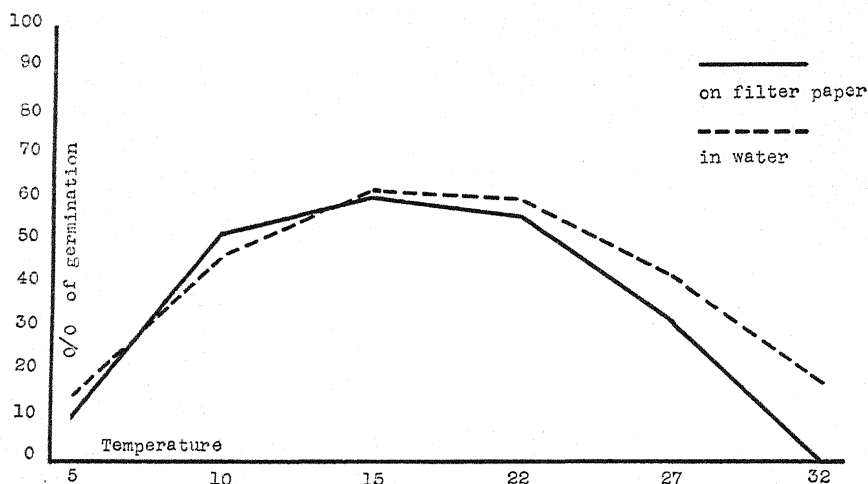
Variety Name	Temperature	No. Seeds Tested	In Water					On Paper				
			Percent Germination after (Weeks)					Percent Germination after (Weeks)				
			2	4	6	8	10	2	4	6	8	10
Dreer's Monarch..	5° C.	2 x 100	0	0	1.0	17.5	35.0	0	0	2.0	21.5	27.5
	10° C.	2 x 100	0	19.5	55.0	62.0	65.0	0	36.5	53.5	61.5	63.0
	15° C.	2 x 100	0	34.0	63.0	67.5	67.5	14.0	50.0	56.5	58.5	59.5
	22° C.	2 x 100	0	0	0	0	0	1.0	1.0	1.0	1.0	1.0
	27° C.	2 x 100	0	0	0	0	0	0	0	0	0	0
Schumacher.....	5° C.	2 x 100	0	0	4.0	40.5	81.5	0	0	60.5	83.5	84.0
	10° C.	2 x 100	0	13.5	66.5	88.0	89.5	4.0	91.0	94.5	94.5	94.5
	15° C.	2 x 100	0	9.5	76.5	86.0	86.5	48.0	86.5	90.0	91.0	91.0
	22° C.	2 x 100	0	0	0	0	0	6.5	16.0	20.5	20.5	20.5
	27° C.	2 x 100	0	0	0	0	0	0	0	0	0	0

<sup>2</sup> We noticed that the maximum temperature for the germination of celery seeds is raised by the effect of light.

Table 9 and text figure 2 show that celery seeds require more time to germinate in water than on filter paper, but there is no difference in final percentage of germination. One variety germinated slightly better, but the other slightly less in water than on filter paper. The maximum temperature, however, is higher on filter paper.

### Germination of Water Cress (*Roripa nasturtium* Rusby) Seeds

As an additional example of water-plant seeds, the germination of water cress seeds was studied at various temperatures in water and on filter paper. The water in the flask was not changed during the experiment. The results are shown in table 10 and text figure 3.



TEXT FIG. 3. Curves showing germination of water cress seeds in water and on filter paper at various temperatures; curves plotted from table 10.

TABLE 10. *Germination of Water Cress Seeds*

Temperature	No. Seeds Tested	In Water				On Paper			
		Percent Germination after				Percent Germination after			
		4 Days	9 Days	15 Days	21 Days	4 Days	9 Days	15 Days	21 Days
5° C.	2 x 100	0	0	3.5	14.5	0	0	0.5	10.0
10° C.	2 x 100	0	21.5	44.5	47.0	0	18.0	41.0	52.0
15° C.	2 x 100	26.0	57.5	60.0	61.5	13.5	47.0	55.0	59.5
22° C.	2 x 100	35.0	58.5	59.0	59.5	20.0	46.0	53.0	56.0
27° C.	2 x 100	7.5	38.5	42.0	42.0	12.5	20.0	26.5	32.0
32° C.	2 x 100	2.5	10.5	16.5	17.5	0	0	0	0
10-22° C.	2 x 100	2.5	44.0	56.0	57.5	0	54.5	61.5	62.0
10-27° C.	2 x 100	2.5	45.0	54.5	55.5	0	49.0	60.0	62.0
10-32° C.	2 x 100	9.5	54.5	61.0	62.5	0	47.5	59.0	60.5
10-38° C.	2 x 100	0	30.5	47.0	47.5	0	0.5	11.5	16.0
15-27° C.	2 x 100	28.5	51.5	52.5	52.5	28.5	50.5	55.5	57.5
15-32° C.	2 x 100	28.5	54.5	57.5	58.0	24.5	49.5	52.5	53.5
15-38° C.	2 x 100	13.0	50.5	55.0	56.5	0	22.0	34.0	36.5
22-32° C.	2 x 100	36.0	56.5	58.0	58.0	26.0	45.0	50.0	53.0
22-38° C.	2 x 100	26.5	51.5	53.0	54.0	0	20.5	29.5	30.0

Table 10 and text figure 3 show that water cress seeds germinate better in water than on filter paper when the temperature is higher than the optimum. This is in agreement with the observations made on white clover seeds.

#### SUMMARY

1. Out of 78 genera of 24 families, 43 genera germinated in water. Even some of the 35 genera which did not germinate under the conditions of the experiment germinated if fewer seeds were placed in the flask of water.

2. Amongst 43 genera which germinated in the water, 18 genera showed no decided difference between the germination in the water and that on filter paper, and 2 genera germinated better under water than on the paper.

3. The ability to germinate under water obtained more generally for small seeds, and was not related to phylogeny or to the kinds of reserve material in the seeds.

4. Out of 21 kinds of seeds which germinated well in distilled water, 20 kinds germinated in boiled distilled water covered with paraffin oil.

5. Several seeds which did not germinate in water were able to germinate when the water was in contact with pure oxygen instead of with air.

6. White clover (*Trifolium repens*) seeds germinated as well in water as on filter paper at optimum temperature (15° C.), but gave nearly 10 times as large percentage of germination in water as on filter paper at 32° C. and at 38° C.

7. Treatment or removal of seed coats was effective in increasing the germination of white clover seeds on filter paper at 38° C., but better results were obtained in water with the seeds treated in the same way.

8. White clover seeds are able to germinate in boiled water sealed with paraffin oil, but are not able to germinate in a vacuum at the optimum temperature. They germinate better in a sealed air chamber over water than in common Petri dishes at 32° C., and still better germination was observed in 60-percent H<sub>2</sub> mixture with air, but 60-percent N<sub>2</sub> mixture was not as favorable as air.

9. Sweet clover (*Melilotus*) seeds germinate equally well in water and on filter paper at high or low temperatures, but they germinate much more quickly in water at high temperature than on the paper.

10. The seeds of red clover (*Trifolium pratense*) and alfalfa (*Medicago sativa*) germinate equally well at the optimum temperature in water and on filter paper, but germination is poorer in water than on paper at temperatures higher than the optimum.

11. Celery (*Apium graveolens*) germinates more slowly in water, but shows no difference between water and filter paper in final percentage of germination. The maximum temperature, however, is higher on filter paper than in water.

12. The seeds of water cress (*Roripa nasturtium* Rusby) germinate

better in water than on filter paper when the temperature is higher than the optimum (15° C.). These seeds also have a higher maximum temperature in water.

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# EFFECT OF ALTERNATING TEMPERATURES UPON THE GERMINATION OF SEEDS

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## INTRODUCTION

Beneficial effects of daily alternations of temperatures in hastening and increasing the percentage of germination of seeds of several species of plants have been proved by many investigations. Alternations of temperatures have also been long used for the practical testing of such seeds as respond to these conditions.

It has been shown that nitrogen compounds and light favor the germination of some seeds that respond to alternation of temperatures. These are often most effective if applied in combination with the alternation of temperatures. In some seeds the need of alternating temperatures is entirely imposed by the seed coats, and this need disappears if the coats are broken.

Since Harrington (7) has recently given an excellent review of the literature and a statement of the theories regarding the effects of alternating temperatures upon the germination of seeds, these points need not be discussed further in this paper, except for such points as naturally need consideration in the discussion of results.

The work here reported includes the following extensions of previous work done on the effect of alternating temperatures upon the germination of seeds: a greater variety and range of alternations, especially the use of 5°, 10°, and 15° C. as the lower temperatures; better controlled temperatures; the effect of the alternations under water and in reduced oxygen pressure, as well as on moist substrata in the usual oxygen pressures; studies of the several factors accessory to alternate temperatures; and the use of seeds of *Typha latifolia* and *Berberis Thunbergii*, not before known to respond to alternating temperatures.

## EXPERIMENTAL METHODS

The following seeds were used in the experiments: Bermuda grass (*Cynodon dactylon* (L.) Pers.), Canada blue grass (*Poa compressa* L.), celery (*Apium graveolens* L.), cat-tail (*Typha latifolia* L.), and Japanese barberry (*Berberis Thunbergii* DC.). All the seeds used were harvested in 1923. In many cases the experiments were run both in the spring and in the

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autumn of 1924, so as to observe the effects of the length of the period of dry storage upon germination behavior.

The seeds were placed in constant-temperature ovens regulated to temperatures of  $10^{\circ}$ ,  $15^{\circ}$ ,  $22^{\circ}$ ,  $27^{\circ}$ ,  $32^{\circ}$ , and  $38^{\circ}$  C. The alternations were secured by daily transfer from one constant temperature to another. This means a rather sudden heating or cooling, depending on the transfer made.

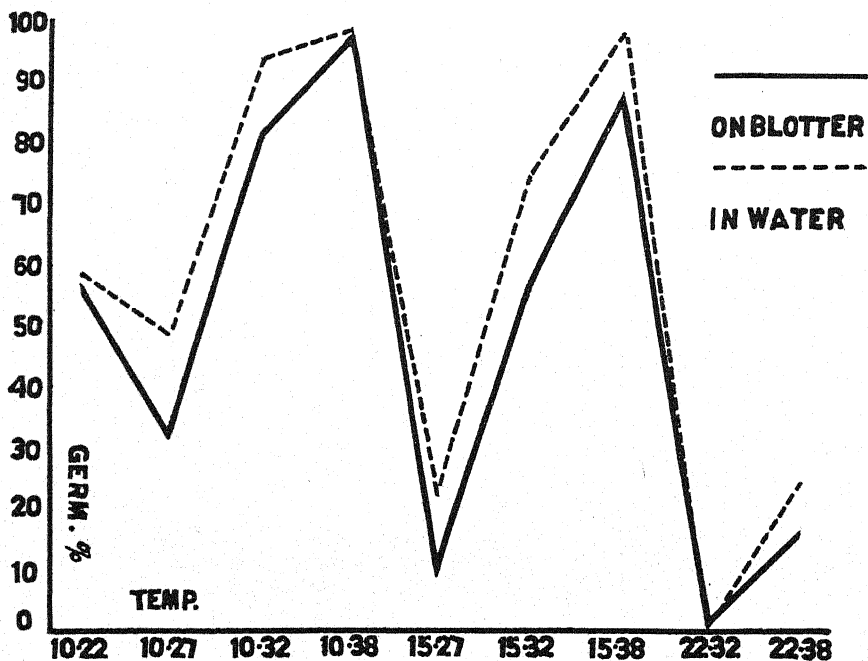
Two types of germination chambers were used: (1) seeds were placed on moist filter paper in Petri dishes, and (2) seeds were kept at the bottom of 100-cc. Erlenmeyer flasks filled with distilled water.

Generally the exposure to the higher temperatures was for 6 hours, and to the lower for 18 hours; but some experiments were carried out to compare the effect of the reverse condition, 18 hours at high temperature and 6 hours at low temperature; and in one experiment a series of duration periods extending from 40 minutes to 8 hours was tried.

### EXPERIMENTAL RESULTS

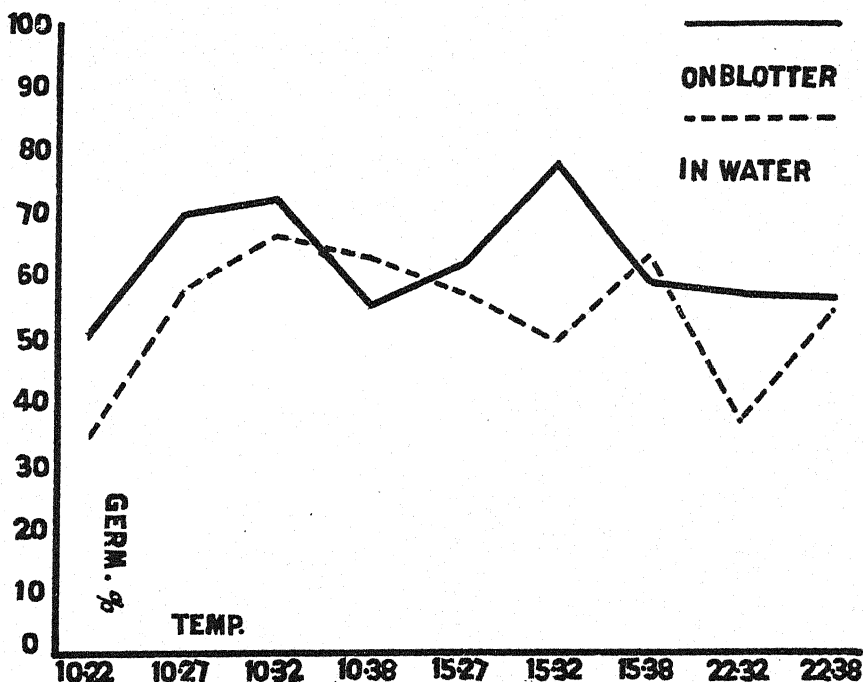
#### Effect of Different Combinations of Alternating Temperatures

On account of lack of space, it was found impossible to include tables showing all the results obtained. In place of them, graphs are herewith presented. The original data can be read with sufficient accuracy from these graphs.



TEXT FIG. 1. Effects of different pairs of alternating temperatures on germination of seeds of Bermuda grass (*Cynodon dactylon* (L.) Pers.).

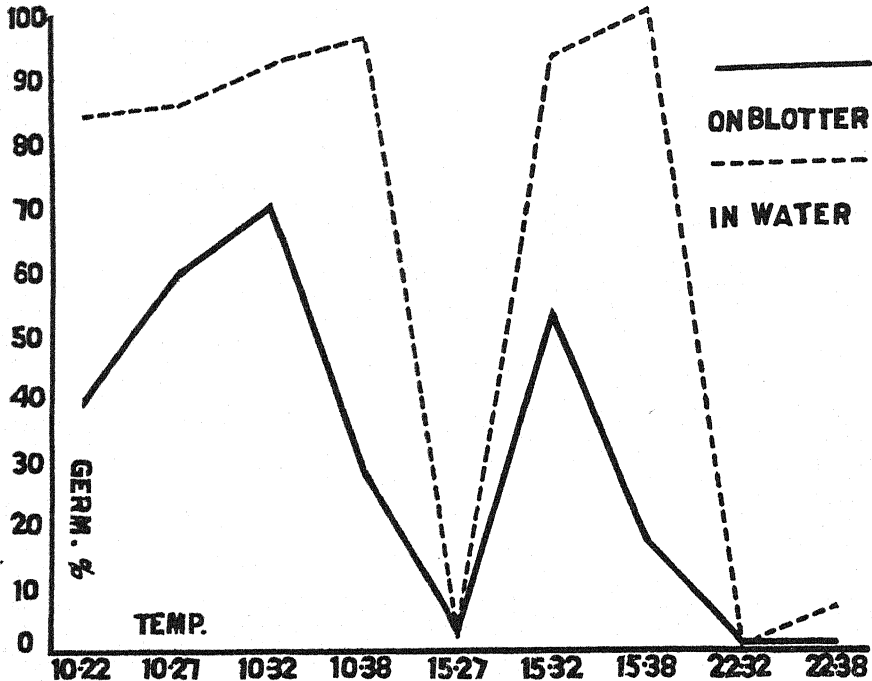
*Bermuda Grass.* Text figure 1 shows the results with nine pairs of alternating temperatures. Bermuda grass does not germinate in constant temperatures in darkness, but it responds well to alternation of temperatures, especially when there is a relatively wide range between the two temperatures used in the daily alternations. Of the nine alternations used with the 6-hour daily period at the higher temperatures,  $10^{\circ}$ – $38^{\circ}$  C. was most effective, and  $10^{\circ}$ – $32^{\circ}$  C. and  $15^{\circ}$ – $38^{\circ}$  C. were slightly less effective. At alternate temperatures Bermuda grass seeds germinate about equally well under water and on moist filter paper. This is probably because these seeds are indifferent to the wide range of oxygen supply represented by these two extremes. As later experiments show, the seeds contain some oxygen and there is some in the distilled water.



TEXT FIG. 2. Effects of different pairs of alternating temperatures on germination of seeds of Canada blue grass (*Poa compressa* L.).

*Canada Blue Grass.* The results are shown in text figure 2. Canada blue grass seeds are not such strict requirers of alternate temperatures in darkness as are Bermuda grass seeds. They gave 12.5 percent germination at  $22^{\circ}$  C. constant, while the best alternations gave only 77.5 percent. The alternation  $15^{\circ}$ – $32^{\circ}$  C. was very effective. The alternation  $15^{\circ}$ – $38^{\circ}$  C. was also effective, giving better germination in one case than other alternations, although  $38^{\circ}$  C. has previously been supposed to be too high for

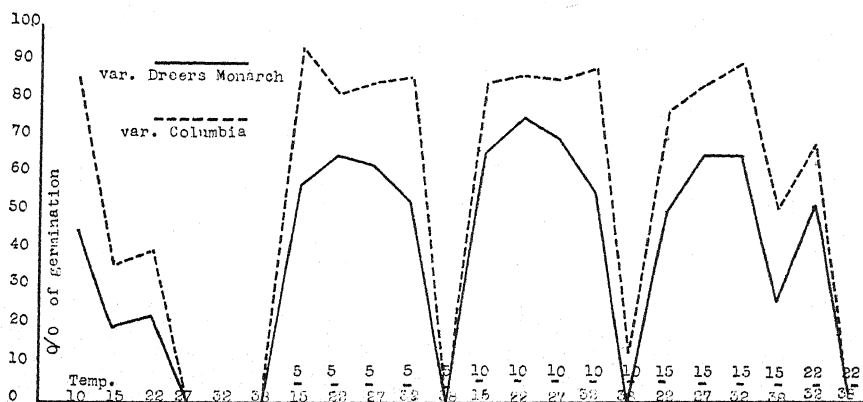
the germination of these seeds. The submerged condition is not highly favorable for the germination of blue grass seeds, probably because of low oxygen supply. Alternation of temperatures, however, has very favorable effects upon the germination of the seeds even under water.



TEXT FIG. 3. Effects of different pairs of alternating temperatures on germination of seeds of cat-tail (*Typha latifolia* L.).

*Cat-tail.* The results are shown in text figure 3. Cat-tail seeds, like Bermuda grass seeds, are decided requirers of alternate temperatures in darkness. The alternations  $10^{\circ}$ – $32^{\circ}$  C. and  $15^{\circ}$ – $32^{\circ}$  C. were favorable, while  $15^{\circ}$ – $27^{\circ}$  C. and  $22^{\circ}$ – $32^{\circ}$  C. were not. At favorable alternating temperatures cat-tail seeds germinate much better under water than they do on moist filters. This result is due, at least in part, to the fact that the water reduces the supply of oxygen to the seeds, as shown in a paper in the present issue of this JOURNAL on "The favorable effect of reduced oxygen supply upon the germination of certain seeds." Cat-tail seeds are sensitive to light, nitrogen compounds, and reduced oxygen pressures. To get best germination of intact seeds, one or more of these conditions must be combined with alternating temperatures. Rupturing of coats removes the necessity of all these conditions and gives good germination at constant temperatures in darkness, and at ordinary atmospheric oxygen concentration with distilled water.

*Celery.* The results of germination tests on moistened filter paper are shown in text figure 4. Celery responds well to a great number of alter-



TEXT FIG. 4. Effects of different pairs of alternating temperatures on germination of seeds of celery (*Apium graveolens* L.); seeds on moist filter papers.

nations. It also germinates fairly well in low constant temperatures, but not at all in higher constant temperatures. Celery seeds germinate somewhat better under water than on moist filter papers, both at low constant temperatures and at alternating temperatures.

#### Effect of Varying Duration of Exposure to each Temperature in the Alternation

Table 1 shows the effect produced upon total germination and upon time required for germination by varying the time the seeds were exposed to the low and high temperatures.

TABLE 1. *Bermuda Grass Seeds at Daily Alternating Temperatures, 15° and 38° C.; Duplicate of 100 Seeds for each Alternation; Period of Experiment, May 22 to June 21; Glumes Intact; in Darkness*

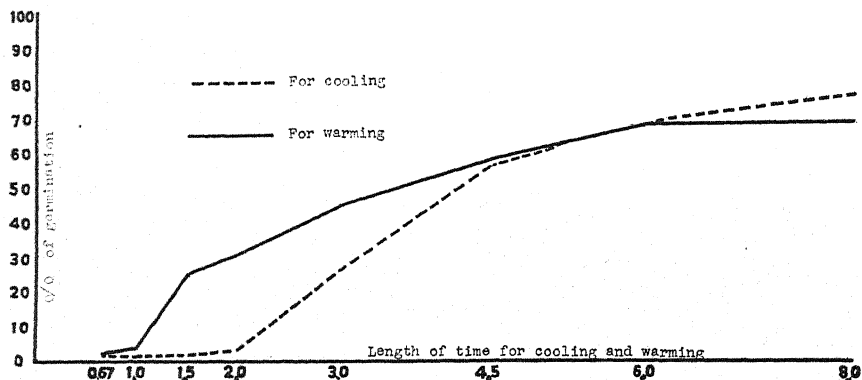
##### With the Short Period at the Higher Temperature

Length of time at higher temperature.....	40 min.	60 min.	1.5 hr.	2.0 hr.	3.0 hr.	4.5 hr.	6.0 hr.	8.0 hr.	15° const.
Percent germination after 30 days.....	2.0	3.5	25.0	30.5	44.5	58.0	67.5	68.0	0
Average number of days for germination.....	21.8	13.3	14.2	14.4	11.7	10.3	12.1	13.5	—

##### With Short Period each Day at Lower Temperature

Length of time at lower temperature.....	40 min.	60 min.	1.5 hr.	2.0 hr.	3.0 hr.	4.5 hr.	6.0 hr.	8.0 hr.	15° const.
Percent germination after 30 days.....	1.5	1.0	1.5	3.0	25.5	55.5	68.0	76.5	—
Average number of days for germination.....	11.7	4.0	9.0	10.2	11.6	12.0	11.8	11.1	—

There was little difference in the effectiveness whether the 18-hour or the 6-hour daily period was at the higher or lower temperature, the principal requirement being that the daily period of exposure to either high or low temperature must be at least 4.5–8 hours.



TEXT FIG. 5. Effects of duration of exposure to the higher and lower temperatures of the alternations; lower temperature 15° C., higher 38° C.; Bermuda grass (*Cynodon dactylon* (L.) Pers.).

Records were also kept of the germinations that took place during each exposure period in order to determine whether the germinations occurred mainly at the higher or at the lower temperature of each alternation. More germinations occurred at the high temperature than at the low, even when the length of time at the higher temperature was only one third of that at the lower temperature.

A daily period of cooling was much less effective than a daily period of warming, when the duration of the cooling or warming was less than 3 hours. A 2-hour daily period of warming gave 30.5 percent germination, while a 2-hour daily period of cooling gave only 3 percent germination.

#### Effect of Nitrogen Compounds upon Germination

*Bermuda Grass.* Preliminary tests were run with duplicates of 100 seeds for each test, at 32° C. constant with 1, 0.1, 0.04, 0.02, 0.0133, and 0.01 *N* solutions of KNO<sub>3</sub>, NaNO<sub>3</sub>, and NaNO<sub>2</sub> on filter paper. Normal and one tenth normal NaNO<sub>2</sub> retarded germination, normal and 0.04 *N* KNO<sub>3</sub> and NaNO<sub>3</sub> did not affect germination. All the other concentrations of these salts increased germination. After 12 days the average germination for all effective concentrations was 15.5 percent, while the checks averaged 3 percent. At the end of 12 days all seeds were transferred to the daily alternating temperature 22°–32° C., with the 6-hour period at the higher temperature. Under this condition all concentrations of the salts, except 1.0 *N* NaNO<sub>2</sub>, gave a higher percentage of germination than the checks.

TABLE 2. *Effect of Various Nitrogen Compounds at Constant and Alternating Temperatures; Experiment Started in February and Ran for 30 Days; Germinators were Filter Paper Moistened with Distilled Water or Solutions; Duplicates of 100 Seeds in each Condition; in the Daily Alternations the 6-hour Period was at the Higher Temperature; in Darkness*

	Temperature Conditions	27° C.	32° C.	38° C.	22-32° C.	22-38° C.
Cont. I (dist. water)	Percent germination after 30 days.....	0.5	0.5	0.5	55.5	72.5
	Average number of days for germination.....	4.0	4.0	4.0	8.2	7.7
Cont. II (dist. water)	Percent germination after 30 days.....	0.5	0.5	1.0	37.5	68.5
	Average number of days for germination.....	2.0	4.0	3.0	8.4	8.8
$\frac{1}{100}$ N. $\text{KNO}_3$	Percent germination after 30 days.....	0	2.0	2.0	95.0	99.0
	Average number of days for germination.....	—	3.0	2.8	4.5	4.8
$\frac{1}{100}$ N. $\text{NaNO}_3$	Percent germination after 30 days.....	0.5	0	3.5	96.0	95.0
	Average number of days for germination.....	4.0	—	4.4	4.6	4.4
$\frac{1}{100}$ N. $\text{NH}_4\text{NO}_3$	Percent germination after 30 days.....	0	0	0.5	93.0	94.5
	Average number of days for germination.....	—	—	4.0	5.4	4.6
$\frac{1}{100}$ N. $\text{HNO}_3$	Percent germination after 30 days.....	1.0	3.5	2.5	93.0	94.0
	Average number of days for germination.....	9.5	5.1	3.2	5.0	4.5
$\frac{1}{100}$ N. $\text{NaNO}_2$	Percent germination after 30 days.....	0.5	1.5	1.0	88.5	93.5
	Average number of days for germination.....	12.0	2.0	2.0	5.9	4.7

After 30 days all lots that were at constant temperatures, and hence showed poor germination, were subjected to the alternation 22°-38° C. Nitrate-treated seeds germinated over 90 percent within 6 days (the seeds that had been kept at 38° C. germinating more quickly than the seeds that had been kept at 27° C.).

It is evident that nitrate and nitrite in combination with the rather unfavorable alternations 22°-32° C. and 22°-38° C. are very effective in increasing both the rate and the percentage of germination of Bermuda grass seeds.

*Cat-tail.* Nitrates cause a marked increase in the germination of cat-tail seeds under water (reduced oxygen pressure) at the constant temperature  $27^{\circ}$  C., but they do not increase the percentage of germination of these seeds on filter papers (full oxygen pressure of the atmosphere) at the same temperature.

### Effect of Combination of Alternating Temperatures, $\text{KNO}_3$ , and Light upon Germination

*Bermuda Grass.* Seeds were first kept for 30 days at constant temperature (room temperature), some lots in darkness and some in light. In each case duplicates of 100 seeds were run in 100-cc. flasks containing 25 cc. of water or of  $\frac{N}{100}$   $\text{KNO}_3$  solution. Not more than 1.5 percent germination was obtained in any case. Similar lots treated in the same way, except that they were alternated between room temperature for 10 hours and  $38^{\circ}$  C. for 14 hours, gave 97.5 percent germination in light and 41.5 percent in darkness.

The seeds that had failed to germinate at constant temperature in light, in darkness, in water, or in  $\frac{N}{100}$   $\text{KNO}_3$  were then alternated between room temperature and  $38^{\circ}$  C. In 3 days the germination was as follows: in water + light, 52 percent; in water + darkness, 7.5 percent; in nitrate + light, 90.5 percent; in nitrate + darkness, 42 percent. After 18 days the germination in the same order was 80.5 percent, 92.5 percent, 96+ percent, 96+ percent.

The favorable effects of N compounds and light on the germination are clear. They increase both the speed and the percentage of germination. They are not as highly effective for germination, however, as are alternating temperatures. They function mainly in combination with alternating temperatures.

*Cat-tail.* Germination was tested under the following sets of conditions: in darkness at the constant temperatures of  $22^{\circ}$ ,  $27^{\circ}$ , and  $32^{\circ}$  C.; in darkness at the alternating temperatures  $22^{\circ}$ – $32^{\circ}$  C.; at room temperature in light and in darkness; one series of all of the above mentioned placed in Petri dishes on moist filter paper, and a duplicate in 100-cc. Erlenmeyer flasks, with 25 cc. of water. In each case duplicates of 100 seeds were used.

At the end of 18 days germination did not exceed 2 percent at any constant temperature in darkness either in Petri dishes or in water; it was 7 percent at alternating temperatures in Petri dishes, but amounted to 88.5 percent at alternating temperatures in water; at room temperature in light the germination was 29.5 percent in Petri dishes, 76 percent in water; but in darkness not more than 1 percent germinated in either Petri dishes or water.

After 18 days all seeds kept at  $22^{\circ}$ ,  $27^{\circ}$ , and  $32^{\circ}$  C. were subjected to

the alternation between 22° and 32° C. for 18 days in darkness. They gave 70 to 83 percent germination in water, but less than 3 percent in Petri dishes. After 18 days the flasks and dishes were transferred to the greenhouse with exposure to direct rays of the sun, where the daily temperature change was considerable. The seeds which did not germinate in the first and the second conditions began to germinate and gave a final germination of over 80 percent in the Petri dishes. The seeds kept in the dark by the window were subjected to light after 18 days. They started to germinate in this new condition, but did not exceed 9 percent even in water. They were finally put for another 18 days in the greenhouse, where they gave 81.5 percent additional germination in water, and 22 percent in the Petri dishes.

The effect of nitrate upon the germination of cat-tail seeds was as follows: In Petri dishes with filter paper moistened with nitrate solutions, germination was no better than on filter paper moistened with water; but when the seeds were immersed in the nitrate solution, the results after 18 days were, for  $\frac{N}{50}$  KNO<sub>3</sub>, 23.5 percent; for  $\frac{N}{100}$  KNO<sub>3</sub>, 59.0 percent; for distilled water, 1.5 percent, at 27° C. in darkness.

*Canada Blue Grass.* The results with this species are shown in table 3.

TABLE 3. *Effect of Alternating Temperatures, Light, and KNO<sub>3</sub> upon Germination of Canada Blue Grass Seed on Moist Filters in Petri Dishes*

Treatment	Percent Germination after 21 Days	
	At Constant Temperature 30° C.	At Alternating Temperature 22°-30° C.
Light + H <sub>2</sub> O.....	30.5	72.0
Darkness + H <sub>2</sub> O.....	5.0	38.5
Light + $\frac{N}{50}$ KNO <sub>3</sub> .....	60.5	95.0
Darkness + $\frac{N}{50}$ KNO <sub>3</sub> .....	21.5	83.0
Light + $\frac{N}{100}$ KNO <sub>3</sub> .....	68.0	94.5
Darkness + $\frac{N}{100}$ KNO <sub>3</sub> .....	11.5	81.0

These results show that light and nitrate are more effective for the germination of Canada blue grass than for Bermuda grass seeds. Thus, over 60 percent of the seeds germinated at constant temperature in light with nitrate. None of the three factors alone is highly effective in bringing about good germination of the seeds. To get maximum effect, the three factors must be used in combination.

#### Effect of Treatment with Concentrated Sulfuric Acid upon Germination of Bermuda Grass and Cat-tail Seeds

*Bermuda Grass.* Seeds of Bermuda grass with glumes removed were treated with concentrated sulfuric acid for 1.5, 3, 4, 5, 6, 7.5, and 9 minutes.



After thorough washing they were put to germinate on moist filter papers at 27° C. in darkness. Within 9 days seeds treated longer than 3 minutes gave 70 to 80 percent germination, while those treated 1.5 minutes gave 45 percent germination and the checks gave 1 percent. Bermuda grass seeds that had been previously soaked for 5 days had a portion of the endosperm cut away at the distal end of the embryo. These gave 86.6 percent germination in 9 days at 27° C. in darkness. Similar results were obtained with seeds that were not previously soaked. A partial removal of the coats of Bermuda grass obviates the necessity of light and alternating temperatures for germination.

*Cat-tail.* The effect of treatment with concentrated sulfuric acid is shown in table 4.

TABLE 4. *Tested at 27° C. in Darkness; Duplicates of 100 Seeds for each Condition*

	Time	Percent Germination after Following Length of Treatment				
		20 sec.	40 sec.	60 sec.	80 sec.	0 sec.
Percentage germinating on wet filter paper in Petri dishes.....	6 days	0.5	32.0	7.0	1.0	1.0
	12 "	0.5	38.0	7.0	2.5	1.0
	18 "	1.5	43.0	7.5	2.5	2.0
	24 "	1.5	44.0	7.5	2.5	2.0
Percentage germinating under water.....	6 days	0.5	45.5	9.5	2.5	3.0
	12 "	0.5	62.0	10.0	2.5	2.0
	18 "	10.0	68.5	10.0	2.5	2.5
	24 "	10.0	68.5	10.5	2.5	2.5

At the close of this experiment the seeds in the flask were transferred to the greenhouse. Within 2 weeks the seeds treated 20 seconds gave 90 percent and the checks 93.5 percent additional germination. The seeds treated 40, 60, and 80 seconds gave 3, 1, and 0.5 percent, respectively, additional germination. A partial removal of the seed coats of these seeds by sulfuric acid treatment increases greatly the germination at a constant temperature, but there is a narrow margin between the treatment that partially removes the coat effects and that which injures the seed.

#### Effect of Mechanical Opening of Seed Coat upon Germination of Cat-tail

Cat-tail seeds are very small, and in shape resemble a slender cone. The large end of the cone is covered with an inset cap with a little knob at the center. When germination occurs, the embryo pushes the cap off and continues to elongate at that end. With some practice under a dissecting microscope it is possible to remove the cap by holding the seed with one needle and gently pressing on the cap with another needle. The results of germinations with coats thus broken are given in table 5.

TABLE 5. *Effect of Breaking Seed Coat upon Germination of Cat-tail Seeds; 25 Seeds in each Condition; Darkness*

	Time	Percent Germination at the Following Temperatures						
		5°	10°	15°	22°	27°	32°	38°
On filter paper in Petri dishes. . . .	5 days	Starting	Starting	96	96	96	88	100
Under water. . . . .	5 days	Starting	Starting	100	96	80	84	96

At 38° C. there was little growth following germination. At 10° C. after 12 days there was 88 and 96 percent germination in Petri dishes and flasks respectively. At 5° C. no germination occurred in 12 days. At 15°, 22°, and 32° C. rapid growth followed germination.

The need for light, reduced oxygen pressures, alternating temperatures, and nitrate solutions for the germination of cat-tail seeds disappears when the coats are broken. These needs are apparently imposed by the seed coats. Whether these conditions modify the coats and make them easier to break, or whether they act upon the embryos, giving them greater expanding pressure for breaking the coats, has not yet been determined.

#### Effect of Alternating Temperatures upon the Germination of Berberis Seeds

Three different collections of *Berberis Thunbergii* (numbered I, II, and III), and one collection of *B. vulgaris* seeds were tested at various constant and alternating temperatures. These tests were made in Petri dishes on moist filter paper. Germination counts were made at the end of 51 days except in the case of *B. Thunbergii* III, which was counted after 40 days. The results are shown in table 6.

TABLE 6. *Effect of Alternating Temperatures on Germination of Berberis Seeds; on Moist Filters; 100 Seeds per Culture*

Percent Germination				
At Constant Temperatures				At Alternating Temperatures
Temperature	<i>B. Thunbergii</i>			<i>B. vulgaris</i>
	I	II	III	
5° C.	64	64	3	0
10° C.	4	40	6	16
15° C.	0	0	1	0
22° C.	0	0	0	16
27° C.	0	0	0	4
32° C.	0	0	0	0
Temperature	<i>B. Thunbergii</i>			<i>B. vulgaris</i>
	I	II	III	
10°-22° C.	100	100	92	84
15°-32° C.	80	92		68
22°-32° C.	24	44	3	36
22°-38° C.	4	0		4
10°-38° C.	16	8		0
5°-10° C.	52	68		24
5°-15° C.	96	84	62-	88
5°-22° C.	100	96	83	72
5°-32° C.	44	56	26-	52
0°-22° C.	0	0		12

# Effect of Low-temperature Storage upon the Later Germination of *Berberis* at Constant and Alternating Temperatures

Seeds of *Berberis* were stored on moist filters from March 10 to April 11 at laboratory temperature and at temperatures of 0°, 5°, 10°, and 15° C. for the purpose of after-ripening previous to germination tests. They were then placed in germinators kept at the constant temperatures of 10°, 15°, 18° C., and at an alternating temperature (10° C. for 18 hours and 22° C. for 6 hours). The effect of this treatment upon germination after 40 days is shown in table 7.

TABLE 7. *Effect of Low-temperature Storage upon Subsequent Germination of Berberis at Constant and at Alternating Temperatures; on Moist Blotters; 100 Seeds per Culture*

Previous Moist Storage Temperature for 32 Days	Germination Temperature	Percent Germination		
		<i>B. Thun.</i> I	<i>B. Thun.</i> II	<i>B. vulgaris</i>
0° C.	10° C.	84	92	8
	15° C.	32	28	52
	18° C.	12	12	24
	10°-22° C.	100	92	76
5° C.	10° C.	96	96	40
	15° C.	68	60	92
	18° C.	84	44	72
	10°-22° C.	100	92	92
10° C.	10° C.	16	36	16
	15° C.	0	4	52
	18° C.	0	0	52
	10°-22° C.	96	96	88
15° C.	10° C.	0	0	0
	15° C.	0	0	20
	18° C.	0	0	8
	10°-22° C.	—	—	—
Room	10° C.	60	—	28
	15° C.	0	0	64
	18° C.	0	—	36
	10°-22° C.	96	92	80

The data in table 7 show that (a) alternate temperatures (10-22° C.) favored germination; (b) 32 days of storage on moist filter paper at 0° C. or at 5° C. was markedly more favorable for subsequent germination than storage in similar conditions at 10°, 15° C., or room temperature; (c) storage at 5° C. gave better results than storage at 0° C.; this was particularly true with *B. vulgaris*; (d) storage at 10° C. gave much lower subsequent germination than storage at 5° C. in the case of *B. Thunbergii*, but the *B. vulgaris* results were nearly as good at 10° C. storage as at 5° C.; (e) although room-temperature storage was much less favorable for germination than storage at 0° and 5° C., room-temperature-stored seeds could be caused to germinate almost as well as low-temperature-stored seeds provided they

were germinated at alternating temperatures ( $10^{\circ}$ – $22^{\circ}$  C.); (f) of the constant temperatures (for germination, not for storage)  $10^{\circ}$  C. was best for *B. Thunbergii* and  $15^{\circ}$  C. for *B. vulgaris*.

### Effect of Alternating Temperatures upon Germination of Low-temperature-stored Berberis Seeds

Since storage at  $5^{\circ}$  C. for 40 days was shown to favor the later germination of Berberis seeds, a test was run to determine more closely the favorable pair of alternating temperatures for the germination of Berberis seeds that had been partially after-ripened by storage at  $5^{\circ}$  C. for 40 days. The results are shown in table 8.

TABLE 8.

Temperature	Percent Germination Previous Treat- ment 40 Days at $5^{\circ}$ C. (in Sand)	No Treat- ment
$5^{\circ}$ C.....	75	1
$10^{\circ}$ C.....	75	31
$15^{\circ}$ C.....	33	0
$22^{\circ}$ C.....	13	0
$27^{\circ}$ C.....	11	0
$32^{\circ}$ C.....	10	0
$5^{\circ}$ – $15^{\circ}$ C.....	90	46
$5^{\circ}$ – $22^{\circ}$ C.....	72	28
$5^{\circ}$ – $32^{\circ}$ C.....	36	0
$10^{\circ}$ – $15^{\circ}$ C.....	91	75
$10^{\circ}$ – $22^{\circ}$ C.....	93	90
$10^{\circ}$ – $32^{\circ}$ C.....	84	80
$15^{\circ}$ – $22^{\circ}$ C.....	94	60
$15^{\circ}$ – $32^{\circ}$ C.....	83	87
$22^{\circ}$ – $32^{\circ}$ C.....	12	0

The data in table 8 show that (a) the alternating temperature pairs  $5^{\circ}$ – $15^{\circ}$ ,  $10^{\circ}$ – $15^{\circ}$ ,  $10^{\circ}$ – $22^{\circ}$ ,  $15^{\circ}$ – $22^{\circ}$  C. (and possibly also  $10^{\circ}$ – $32^{\circ}$ ,  $15^{\circ}$ – $32^{\circ}$  C.) were more favorable for the germination of partially after-ripened Berberis seeds than any constant temperature tried; (b) a difference of about  $10^{\circ}$  C. in the alternating pair gave better results than a temperature difference amounting to as much as  $17^{\circ}$  C.; (c) when the lower temperature of the alternating pair was as high as  $22^{\circ}$  C., the favorable effects of the alternation and of the previous low-temperature storage were nearly lost; (d) seeds stored at  $5^{\circ}$  C. germinated better than those not after-ripened at a low temperature; but seeds that were not treated germinated nearly as well as the treated seeds, provided the germination temperature was a favorable alternating temperature, especially  $10^{\circ}$ – $22^{\circ}$  C.

### DISCUSSION

#### Effect of Alternating Temperatures with Distilled Water on Filter Paper as the Germinator

The results of experiments on filter paper with Bermuda grass (*Cynodon dactylon* (L.) Pers.) at six constant and nine alternating temperatures, and

with celery (*Apium graveolens* L.) at six constant and sixteen alternating temperatures, agree in the main with Harrington's work on these seeds (7), although he worked with fewer constant and alternating temperatures.

Bermuda grass seeds give practically no germination at constant temperatures, but give a high percentage of germination at various alternations. Harrington got best germination, nearly 80 percent, at the alternations 15°-35° C. and 20°-35° C. In the work here reported, best germination, over 90 percent, was obtained at alternations 10°-38° C. and 15°-38° C., extremes not used by Harrington.

Celery seeds germinated well at low constant temperatures (86 percent at 10° C.), and there was fair germination at 5° C. The lowest temperature used by Harrington was 15° C., which in this work proved less favorable in some varieties than 10° C. With a rise in the constant temperatures in darkness, germination fell off, so that none occurred above 27° C. Many different alternations gave excellent germination. This was especially true when the lower temperatures in the alternation were 5°, 10°, or 15° C., and the higher temperature was 32° C. or less. The retarding effect of high temperatures is counteracted by alternations. At 22° C. constant, two varieties gave 23 and 40 percent germination respectively, while the same varieties gave 53 and 67 percent respectively at the daily alternation 22-32° C.

The fruit scales retard the germination of *Chloris ciliata* (6), *Avena elatior*, and *Holcus halepensis* (4). This is not true of Bermuda grass.

Vanha (9) found the daily alternation with *Poa pratensis* most effective when the daily high temperature period was short (4 hours). Bermuda grass seeds respond equally well to daily alternations with the high-temperature periods ranging anywhere from 6 to 18 hours. If in the daily alternations the daily period at the low or high temperature is 4 hours or less, the high temperature for the short period is more effective than the low temperature for the short period.

In the daily alternations with Bermuda grass seeds, more seeds germinate during the high-temperature periods than during the low-temperature periods, although the former are only one-third as long. Bermuda grass seeds endure higher germination temperatures than celery and some other seeds studied.

The behavior of Canada blue grass (*Poa compressa* L.) seeds at constant and alternate temperatures is very similar to that found by Harrington for *Poa pratensis* seeds. There is some germination (10 percent or more) at favorable constant temperatures. The germination at favorable alternations runs from 60 to 80 percent.

Cat-tail seeds, like Bermuda grass seeds, germinate less than 1 percent at constant temperatures, but give considerable germination, as high as 70 percent, at favorable alternations.

*Berberis Thunbergii* and *B. vulgaris* seeds respond to various alternating

temperatures. The alternation  $10^{\circ}$ – $22^{\circ}$  C. is especially favorable. These seeds also germinate at low constant temperatures,  $10^{\circ}$ ,  $15^{\circ}$ , and  $20^{\circ}$  C., if they are first after-ripened in a germinator at  $5^{\circ}$  C. This after-ripening also improves the germination at various alternations. The germination at  $5^{\circ}$  C. constant after 2 to 4 months is preceded by after-ripening at this temperature. These seeds responding to after-ripening in a germinator at a low temperature represent a class of seeds needing alternating temperatures that have not been previously mentioned in the literature.

#### Effect of Alternating Temperatures upon Germination of Seeds under Water

Kinzel (8) has studied the effect of light upon the germination of seeds under water, but so far as the writer knows no work has been done upon the effect of alternating temperatures upon the germination of seeds under water.

For all the seeds studied, Bermuda grass, Canada blue grass, cat-tail, and celery, alternating temperatures, compared with constant temperatures, favor germination when the seeds are under water much as they do when the seeds are on filter paper moistened with water.

Bermuda grass seeds at the most favorable alternations germinate somewhat better under water than on filter paper moistened with water, but they show somewhat poorer germination at unfavorable alternations under water than on filter paper. The average for all alternations is about equal under water and on moist filter papers.

At alternating temperatures, Canada blue grass seed germinates much better on moist filter paper than in water; cat-tail seeds germinate much better in water than on moist filter paper; and celery germinates about equally well in both conditions. Water favors the germination of the cat-tail seeds by reducing the oxygen supply to the seeds.

#### Effect of Light and N Compounds upon Germination of Seeds Favored by Alternating Temperatures

The effect of light upon the germination of seeds has been studied by many workers, and has been considered as having close relation to the temperature effects. A review of the literature on the effect of light on germination appears in a recent article by Gardner (4). Gassner (6) and others have shown the effectiveness of N compounds for forcing germination.

Nitrates and nitrites have little effect upon the germination of Bermuda grass seeds at constant temperatures, but they increase the germination at unfavorable alternations both under the solution and on filter paper moistened with the solution. Light was also effective on this seed when acting in combination with poor alternations, but not in favorable alternations. The results obtained with Bermuda grass seeds are similar to those obtained by Gassner (6) for *Ranunculus sceleratus* seeds; in both

cases alternation of temperatures is the highly effective factor, and light and N compounds supplement the effects of alternating temperatures.

The effects of light and nitrogen compounds are much more striking on the seeds of Canada blue grass; they favor the germination of these seeds even at constant temperatures and in both the favorable and the unfavorable alternations. They have a primary effect here, as well as supplementing the effect of alternating temperatures. Nitrates and light both greatly favor the germination of cat-tail seeds even at constant temperatures. Cat-tail seeds kept in a germinator in darkness for a while germinate more poorly when given favorable conditions. As in the case of *Ranunculus* seeds, they take on a darkness rigor.

### Breaking of Seed Coats in Relation to the Effect of Alternating Temperatures

It has been shown that seed coats interfere with germination in various ways; by exclusion of water, in "hard seeds"; by curtailing water-absorption or restricting swelling, in *Alisma* (3); by restricting oxygen supply to the embryos (2); by interfering with carbon dioxide elimination; and perhaps by other means not yet worked out, such as retaining inhibiting agents within the seeds.

Bryan (1) has shown that treating Bermuda grass seeds with concentrated sulfuric acid increases the germination. In a previous paragraph it is shown that both this treatment and mechanical removal of a portion of the fruit coats remove the necessity of alternating temperatures. In the same section it is shown that both sulfuric acid treatment and breaking the coats of cat-tail seeds remove the need of light, reduced oxygen pressures, alternating temperatures, or nitrate solutions for the germination of these seeds. Harrington (7) has shown that Johnson grass seeds, which respond well to alternating temperatures but germinate poorly at constant temperatures, germinate perfectly at various constant temperatures if the coats are broken.

It is not safe to assume, however, that in any of these cases the alternating temperatures and other effective conditions act solely upon the seed coats. Take the simplest assumption, namely, that the expanding force of the embryo or other seed contents is not sufficient to break the coats. The alternating temperatures, or other effective conditions, may either weaken the coats or in some way increase the expanding force of the embryo by modifying the swelling or the growth of the embryo. The coats may impose the need of alternating temperatures in various other ways even in this class of seeds. In other seeds, such as blue grass and celery, breaking the coats does not dispose of the need of alternating temperatures or of other substituting factors.

The little evidence we have indicates that in both classes of seeds mentioned above alternating temperatures have their effects on the embryos.

In an unpublished work reported December, 1924, before the Physiological Section of the Botanical Society of America, W. E. Davis has found that celery seeds show a much greater total rise in catalase activity at alternating temperatures previous to germination than is ever shown at corresponding constant temperatures. He assumes that alternating temperatures as such are modifying the growth behavior of the embryos themselves. The writer has found the same for Bermuda grass seeds, where, in contrast to celery, the coats impose the necessity of alternating temperatures. Upon the whole it seems safe to say, however, that we still have to learn in the main the mechanism, or perhaps the various sorts of mechanisms, by which alternating temperatures and their substituting factors promote germination.

#### SUMMARY

1. Alternating temperatures were effective in germination of seeds of *Cynodon dactylon*, *Poa compressa*, *Typha latifolia*, *Apium graveolens*, and *Berberis Thunbergii*.

2. More seeds of *Cynodon dactylon* started to germinate at the higher temperature of the alternation than at the lower, even though the length of time at the former temperature was only one third that at the latter.

3. Alternating temperatures were necessary and sufficient to germinate intact seeds of *Cynodon dactylon* and *Typha latifolia*. *Poa compressa* seeds germinated about 10 percent at various constant temperatures, and did not give the highest percentage of germination even under the best alternating temperatures.

4. *Apium graveolens* seeds germinated as well at the low temperature (10° C.) as at alternating temperatures. *Berberis Thunbergii* seeds also germinated at low (5° C.) or alternating temperatures. After keeping the latter for 30 days at 5° C., they were able to germinate at higher constant temperatures.

5. *Cynodon dactylon* seeds germinate slightly better in water than on filter paper, and *Poa compressa* seeds germinate slightly less in water than on filter paper when they are under favorable alternating temperatures. *Apium graveolens* seeds germinate to as high percentage in water as on filter paper. *Typha latifolia* seeds germinate much better in water than on filter paper.

6. Light and nitrate as well as nitrite were effective in the germination of *Cynodon* seeds, when applied together with alternating temperatures. The effects of light and nitrate were more striking on *Poa* seeds. They increased the percentage of germination at constant and alternating temperatures. The highest percentage of germination of *Poa* seeds was obtained when these three factors were used in combination.

7. Nitrate did not affect the germination of celery seeds, and light was effective in germination only when unfavorably high temperatures were used.



8. Mechanical treatment or treatment with  $\text{H}_2\text{SO}_4$  (concentrated) was effective in forcing the germination of *Cynodon* and *Typha* seeds at constant temperatures.

9. *Cynodon dactylon*, *Poa compressa*, and *Typha latifolia* seeds germinated in boiled distilled water covered with paraffin oil; but *Cynodon dactylon* seeds and *Typha latifolia* seeds, coats entire or broken, did not germinate in a vacuum.

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# THE FAVORABLE EFFECT OF REDUCED OXYGEN SUPPLY UPON THE GERMINATION OF CERTAIN SEEDS

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(Received for publication December 4, 1925) <sup>1</sup>

## INTRODUCTION

A number of workers (1, 2, 3, 5) have shown that *increasing* the partial oxygen pressure of the atmosphere brings about or improves the germination of intact seeds of several species of plants. Work reported in this paper, however, shows that *reducing* the oxygen pressure has a very beneficial effect upon the germination of intact cat-tail seeds at a variety of temperatures, and a marked effect upon the germination of Bermuda grass seeds.

With both cat-tail and Bermuda grass seeds, diluting the air with nitrogen or hydrogen favored germination. The optimum for cat-tail seeds was obtained by diluting the air with 40 to 80 percent of these gases, and for Bermuda grass with 40 to 60 percent.

Cat-tail seeds germinated much better in atmospheres of one hundredth normal oxygen pressure than in normal oxygen pressure, but chlorophyll-development in the seedlings required greater pressure than this, namely, about 4 percent normal oxygen pressure.

These results must not be taken to mean that reduced oxygen pressure will favor the germination of most seeds. It is probable that relatively few seeds are thus favored in germination.

## EXPERIMENTAL RESULTS

### Effect of Reduced Oxygen Pressures on Germination of Seeds of Cat-tail (*Typha latifolia* L.)

To secure the oxygen pressures desired, Petri dishes without covers, containing the seeds on moist filter papers, were placed on a tripod in a pan. Battery jars were inverted over the Petri dishes and tripods. Water was poured in the pans to give water seals, as well as water to displace the air to be withdrawn. Sufficient air to give the desired mixture was then withdrawn and replaced by hydrogen or nitrogen. All lots were then placed in the greenhouse at temperatures with a daily range from about 15° to about 30° C. The results obtained when hydrogen was mixed with air are shown in table 1 and in text figure 1.

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

TABLE 1. *Effect of Reduced Oxygen Pressure on Germination of Cat-tail Seeds; Air Diluted with Hydrogen Gas; Duplicates of 200 Seeds for each Condition*

Time	Percent Germination						
	Check in Petri Dish*	Check under Sealed Jar†	20% H <sub>2</sub> , 80% Air	40% H <sub>2</sub> , 60% Air	60% H <sub>2</sub> , 40% Air	80% H <sub>2</sub> , 20% Air	90% H <sub>2</sub> , 10% Air
2 days.....	0	1.3	88.0	96.8	95.8	95.5	93.5
4 days.....	0	42.3	94.8	97.5	96.5	97.3	96.3
6 days.....	0	80.8					
8 days.....	1.3	85.5					
10 days.....	3.8	86.0					

\* Seeds in Petri dishes, loosely covered, exposed to air.

† Seeds in air inside inverted battery jars with water seal.

The results obtained when air was replaced by nitrogen instead of hydrogen are shown in table 2 and text figure 2.

TABLE 2. *Effect of Reduced Oxygen Pressure on Germination of Cat-tail Seeds; Air Diluted with Nitrogen Gas; Duplicates of 200 Seeds for each Condition*

Time	Percent Germination						
	Check in Petri Dish*	Check under Sealed Jar†	20% N <sub>2</sub> , 80% Air	40% N <sub>2</sub> , 60% Air	60% N <sub>2</sub> , 40% Air	80% N <sub>2</sub> , 20% Air	90% N <sub>2</sub> , 10% Air
2 days.....	0	0	10.3	49.0	95.5	94.3	89.8
4 days.....	0	42.5	93.8	94.5	97.8	96.3	97.8
6 days.....	0	70.5					
8 days.....	1.3	83.5					
10 days.....	3.8	84.5					

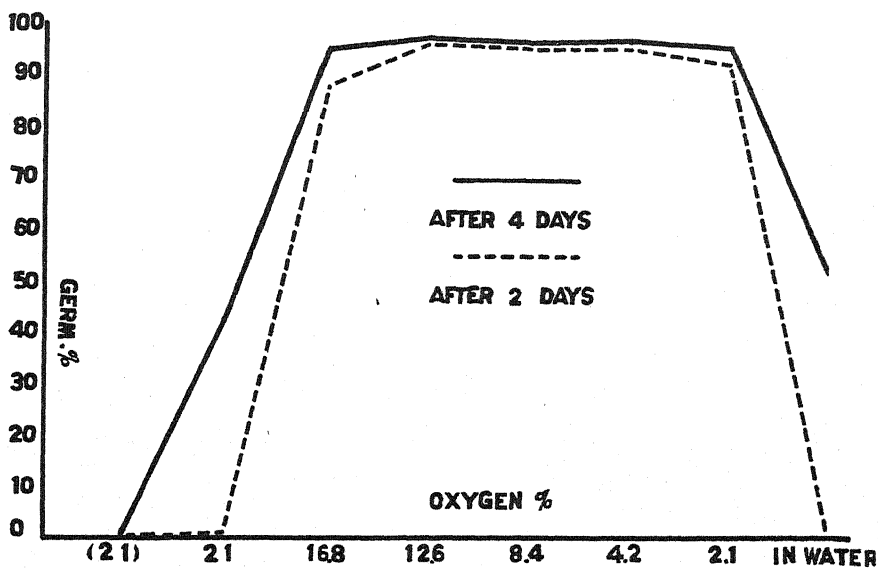
\* Seeds in Petri dishes, loosely covered, exposed to air.

† Seeds in air inside inverted battery jars with water seal.

The results obtained by diluting air with nitrogen were similar to those obtained by diluting with hydrogen. From tables 1 and 2 it is evident that prompt and almost complete germination was obtained with cat-tail seeds on moist filter paper when the partial oxygen pressure of the atmosphere was reduced by additions of hydrogen or nitrogen. Favorable effects appeared with any additions from 20 to 90 percent, the most favorable being with additions of 40 to 80 percent.

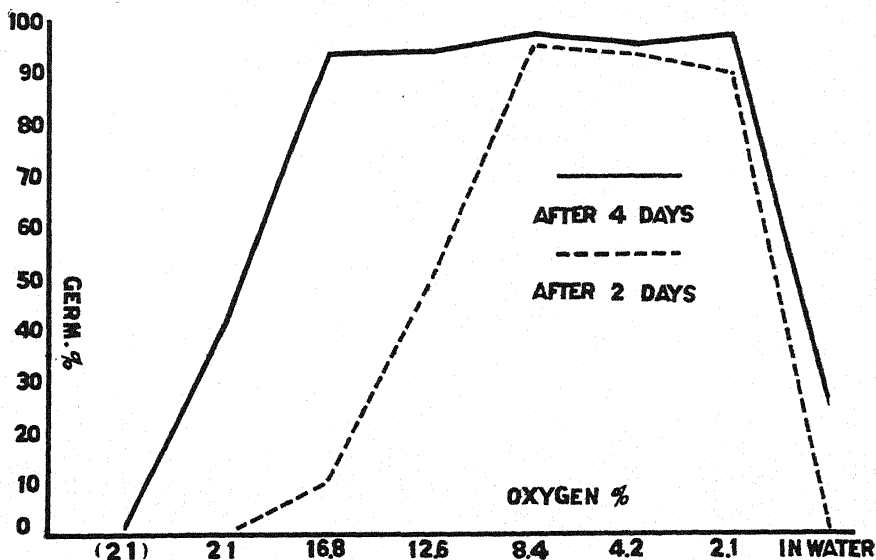
From tables 1 and 2 it can be seen that, in the check lots in Petri dishes loosely covered with easy access to air, the germination did not exceed 4 percent; in the check lot that was placed in air under an inverted battery jar protected from the outside air by a water seal, germination proceeded slowly and finally reached about 85 percent.

Further evidence regarding the effect of sealing the germination chambers was obtained from the following experiment. Two hundred seeds each



TEXT FIG. 1. Effect of reduced oxygen pressure on germination of cat-tail seeds; air diluted with hydrogen gas.

were put on wet filter paper in large Petri dishes, and one set of these dishes was sealed with modeling clay with a little dish of NaOH solution inside; a second set was arranged similarly without the caustic solution and a third set was placed in unsealed Petri dishes. After 10 days in the



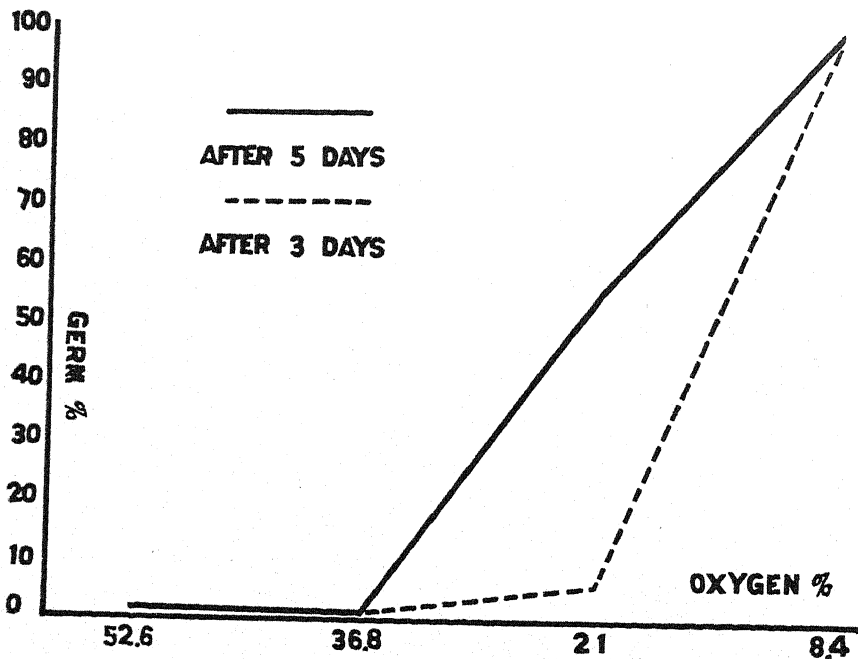
TEXT FIG. 2. Effect of reduced oxygen pressure on germination of cat-tail seeds; air diluted with nitrogen gas.

greenhouse 85.5 percent of seeds germinated in the dishes with NaOH, 94.8 percent in the dishes without, and only 2.8 percent of seeds germinated in the dishes that were not sealed.

The reasons for better germination inside the battery jars than in Petri dishes are not known. That this result is not due to a reduction in the oxygen concentration by the respiration of the seeds is indicated by the fact that the oxygen concentration inside the closed battery jar after 8 days had been reduced only from 20.4 percent to 19.4 percent. It may be that a more favorable alternation of temperature was obtained inside the battery jar. Further work on this point needs to be carried out.

#### Effect of Increased and Decreased Oxygen Pressures on Germination of Cat-tail Seeds

In order to obtain further evidence on the rôle of oxygen in the germination of cat-tail seeds, increased oxygen concentrations, obtained by adding oxygen to air, were compared with decreased oxygen concentrations, obtained by adding nitrogen to air. The results are shown in table 3 and text figure 3. These experiments were performed under battery jars as were the previous ones.



TEXT FIG. 3. Effects of increased and decreased oxygen concentrations upon germination of cat-tail seeds.

TABLE 3. *Comparison of Effects of Increased and Decreased Oxygen Concentrations on the Germination of Cat-tail Seeds; Duplicates of 200 Seeds for each Condition*

Time	Percent Germination			
	40% O <sub>2</sub> Added to Air	20% O <sub>2</sub> Added to Air	Air	60% N <sub>2</sub> Added to Air
3 days.....	1.8	1.3	6.3	97.3
5 days.....	1.8	1.3	54.3	98.3
9 days.....	1.8	1.3	67.5	

Table 3 shows clearly the favorable effect of reduced oxygen concentrations on the germination of cat-tail seeds. The fact that the seeds in the air lot germinated slowly and gave 67.5 percent germination in 9 days shows that some other factor was operating in conjunction with reduced oxygen pressure in forcing germination; but the favorable effect of this factor was nullified, at least during the time of this experiment, by the addition of extra oxygen to the battery jar.

#### Effect of Lower Concentrations of Oxygen on the Germination of Cat-tail Seeds

In another set of experiments under battery jars in the greenhouse, 94 percent of the seeds germinated in 99 percent hydrogen mixture with air after 4 days, but gave white seedlings. In 96 percent hydrogen the germination was as good and the seedlings were yellowish green. Evidently the oxygen supply required for germination is less than that required for chlorophyll-development, as Crocker and Davis (4) found for *Alisma Plantago*.

Can cat-tail seeds germinate in complete absence of gaseous oxygen? The desired number of seeds was placed in a 25-cc. distilling flask with 10 cc. of water. The neck of the flask was sealed, and the side tube was drawn down to a capillary at one region. The side tube was then connected with a high vacuum pump, and the water in the flask was boiled under vacuum in a water bath at 40°-45° C. for 30 minutes, or until about 1 cc. of water remained in the flask. The side tube was then sealed off while still being evacuated and boiled. In consequence of evaporation under the vacuum, the seeds always remained far below the temperature of the bath so that there was no injury from high temperature. The flasks were then put in the most favorable conditions for germination. With cat-tail seeds this involved also breaking the seed coats before sealing in the flasks. No germination was obtained when, by this method, oxygen was removed completely or nearly completely from both water and seeds.

### Effect of Reduced Oxygen Pressures upon Germination of Bermuda Grass Seeds

The effect of reduced oxygen concentrations upon the germination of Bermuda grass (*Cynodon dactylon* (L.) Pers.) was then tested, using the same method that was employed in the experiments with cat-tail seeds. The oxygen concentration was reduced by diluting air with hydrogen. The results are shown in table 4.

TABLE 4. *Effect of Reduced Oxygen Pressures upon Germination of Bermuda Grass; Duplicates of 100 Seeds Used in each Condition*

Time	Percent Germination						
	Check in Petri Dish*	Check under Sealed Jar†	20% H <sub>2</sub> , 80% Air	40% H <sub>2</sub> , 60% Air	60% H <sub>2</sub> , 40% Air	80% H <sub>2</sub> , 20% Air	90% H <sub>2</sub> , 10% Air
6 days.....	1.0	15.5	17.0	26.0	32.0	21.5	10.5
8 days.....	15.5	62.0	73.0	81.5	83.5	75.5	53.0
10 days.....	24.5	73.5	84.5	89.0	91.0	85.0	63.5

\* Seeds in Petri dishes, loosely covered, exposed to air.

† Seeds in air inside inverted battery jars with water seal.

When this experiment was repeated with 60-percent nitrogen and 60-percent hydrogen mixtures with the air, the former gave 89.5 percent and the latter 85.5 percent germination after 10 days as against 55 percent for the checks.

The data in table 4 show: (1) That a reduction in oxygen concentration of air favored the germination of Bermuda grass, although the favorable effect was less marked than for cat-tail seeds; (2) the favorable mixtures were 40-60 percent hydrogen mixed with 60-40 percent air. With 90 percent hydrogen and 10 percent air the germination was not as good as with the checks under the sealed jar receiving no treatment.

### Influence of Seed Coat upon Oxygen Requirement for Germination of Cat-tail Seeds

In the case of cat-tail seeds, at least, the favoring effects of reduced oxygen pressures disappeared when the seed coats were broken. When this was done these seeds germinated well, at various constant temperatures, in oxygen pressures ranging anywhere from 1 to 90 percent oxygen of full atmospheric pressure. The mechanism by which reduced oxygen pressures favor the germination of intact seeds, when the naked embryos are so indifferent to broad variations in oxygen pressure, is not known. It is not safe to assume, however, that the reduced oxygen pressure affects the coats alone, although this may be the case. It may also act upon the

embryo mainly, as is suggested for alternating temperatures in a preceding paper in the present issue of this JOURNAL on the "Effect of alternating temperatures upon the germination of seeds."

### Effect of Reduced Oxygen Pressures on the Germination of Certain Other Seeds

Reduced oxygen pressure also favors the germination of water cress (*Roripa nasturtium* Rusby) and white clover (*Trifolium repens* L.) seeds at 22° C. or above, as shown by the results reported in a paper "Germination of seeds under water," also published in the present number of this JOURNAL. Reduced oxygen pressure does not favor the germination of sweet clover (*Melilotus alba* Desr.) and alfalfa (*Medicago sativa* L.) seeds at any temperature, as shown in the same paper.

### SUMMARY

1. Seeds of cat-tail (*Typha latifolia* L.), which germinate poorly or not at all in air, germinate promptly when the oxygen concentration of the air is reduced by diluting with hydrogen or nitrogen.

2. Favorable concentrations were obtained by diluting the air with 40 to 80 percent (by volume) of hydrogen or nitrogen, approximately 96 percent germination resulting.

3. When the oxygen concentration was increased by adding 20 percent oxygen to air, germination was 1.3 percent; but adding 60 percent nitrogen to air brought about prompt germination (97-98 percent).

4. Cat-tail seeds germinated 94 percent in a 99-percent hydrogen mixture with air, but the resulting seedlings were white, there being insufficient oxygen for chlorophyll-development.

5. When special precautions were taken to remove oxygen completely or nearly completely from the flasks, liquids, and seeds, no germination resulted. A small supply of oxygen is necessary for germination.

6. The favoring effects of reduced oxygen pressures disappear when the seed coats are broken. Under this condition germination proceeds readily in oxygen pressures ranging from 1 percent to 90 percent of the full atmospheric oxygen pressure.

7. Bermuda grass (*Cynodon dactylon* (L.) Pers.) also germinates better when the oxygen pressure of the air is reduced by diluting with hydrogen or nitrogen, although the favorable effect was less marked than for cat-tail seeds; favorable mixtures were obtained by diluting air with 40-60 percent hydrogen or nitrogen.

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## EVAPORATION IN VEGETATION AT DIFFERENT HEIGHTS<sup>1</sup>

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### INTRODUCTION

It is expected that the evaporation will increase as one goes upward from the ground, but the amount varies considerably for different places and different conditions. It was partly with the view of ascertaining the values of such increases in the region about Douglas Lake, the location of the Biological Station of the University of Michigan, that the different pieces of work here reported upon were undertaken. The fact that evaporation increases with increase in height above the ground in marsh vegetation has already been brought out by Yapp<sup>2</sup> and confirmed by Dachnowski<sup>3</sup> and Sherff,<sup>4</sup> in bog and marsh vegetation respectively.

In the present investigation, not only marshes but also shrub and small-tree growths were utilized as experimental ground. The region is differently characterized climatically from that considered in either of the above-cited papers.<sup>5</sup>

### METHODS

Throughout these investigations the normal methods employed in atmometer work have been employed. Standardized Livingston atmometers were utilized for from 40 to 52 days, including the hottest and driest part of the year. In the earlier work cylindrical instruments were used, which were replaced by the spherical type as soon as the latter were manufactured. Readings were taken at the same time with reference to rains, so

<sup>1</sup> Contribution no. 241 from the Botanical Laboratory of Kansas State Agricultural College and a contribution from the Biological Station of the University of Michigan.

<sup>2</sup> Yapp, R. H. Stratification in the vegetation of a marsh. *Annals Bot.* 23: 275-319. 1909.

<sup>3</sup> Dachnowski, A. The vegetation of Cranberry Island and its relations to the substratum, temperature and evaporation. *Bot. Gaz.* 52: 126-150. 1911.

<sup>4</sup> Sherff, E. E. Evaporation conditions at Skokie Marsh. *Plant World* 16: 154-160. 1913.

<sup>5</sup> For figures of daily summer evaporation from the standard in this region consult table VI in the paper entitled:

Gates, F. C. Meteorological data, Douglas Lake, Michigan. *Papers Mich. Acad. Sci. Arts Lett.* 4: 475-489. 1924.

[The *Journal* for February (13: 59-166) was issued February 22, 1926.]

as to maintain as nearly as possible the relative values; but as soon as rain-correcting atmometers were manufactured, they were employed in subsequent work. The atmometer cups were set up in the usual manner and read by filling to a mark which permitted an accuracy of reading to within  $1/2$  cc. in the earlier part of the work and to within  $1/20$  cc. in the later part. Observations in the different sets were made as closely as possible at the same time so as to maintain the same relative values. In some of the sets the atmometers were placed on stakes driven into the ground, making the center of the atmometer 0.3 m. from the actual surface; in other cases poles were arranged so that the atmometers could be quickly and safely brought down for reading and then replaced in the crown of the tree, the evaporating conditions of which were being measured. The actual measurements were usually taken by different graduate students under the author's direction.

Each series will be taken up separately. A general description of the stations will be followed by a statement of the results there obtained.

#### PINE SERIES

This series was run with cylindrical atmometers from July 9 to August 18, 1917, by E. E. Watson in stations in the pine land in the vicinity of the Biological Station. In this series 5 sets were used.

Set no. 1 was run on the brow of a low hill somewhat less than a mile from Douglas Lake in open country kept open in consequence of recurring fires. An atmometer was maintained in the highest tree in this vicinity at a height of 4 meters from the ground, while a second was at the ground. In this case both atmometers were well exposed to the wind, as conditions were very open all around the pine tree. Under such conditions the evaporation at four meters was 1.18 times that at the ground.

Set no. 2 was located on the southeast side of a ridge south of Douglas Lake in an area where there was a generous growth of young aspen sprouts following burning at least three years previously, although previous to that time there had been additional fires. The atmometer was run in the crown of a tree 1.8 meters high, but surrounded by numerous small trees and shrubs of about the same height. Wind drainage down the slope was very obvious. The elevation of the atmometer, 1.8 meters in this case, resulted in an increase in the rate from unity to 1.02.

The third and fourth sets were run a little over a hundred meters from Douglas Lake in a piney area in which there were a considerable number of seedling pines and a fair number of aspens varying to a maximum of about 2.7 meters in height, besides many small trees and shrubs and an abundance of *Pteris*, the latter about 0.8 meter high. In the third set an atmometer was maintained in a pine 1.5 meters in height and showed a rate of 1.61 as compared with unity for the ground. In the fourth set, not far away, the atmometer 0.5 meter high gave 1.30 in comparison with the unit value at the ground.

The unit value as expressed between ground readings varied in the different sets, as was to be expected, since certain sets were in places where the wind had ready access, as in sets 1 and 2, especially, however, in set 1; while sets 3 and 4, near the lake and in much moister air, would not be expected to show so high a rate.

#### ASPEN SERIES

A second and more extensive series was run similarly in the crowns of aspens of different heights in the vicinity of the Biological Station by B. H. Grisemer from July 9 to August 20, 1917.

Set no. 1 was run on the upland a little way back from Douglas Lake over the ridge towards Smith's Bog in an area formerly pine but now covered with aspen except where this was interrupted in consequence of fires. Three pairs of atmometers were run in close proximity to one another. The figures for the ground atmometers of these three pairs gave rates so close together that the figures of the atmometers in the crowns of the aspens indicate an acceleration in the rate of evaporation for at least 1.8 meters, followed by a diminishing increase beyond to at least 3.7 meters. The increase per foot (0.3 meter) varied between 0.71 and 1.16 cc. / hr. Considering the value of the atmometer at the ground in each case as unity, at 0.9 meter the value was 1.16; at 1.8 meters, 1.39; and at a 3.7-meter aspen crown, 1.48.

The fourth pair of atmometers in this set was run near the west end of this ridge, about a kilometer from the other three, in denser vegetation of the same general type. The atmometer in the crown of the aspen 2.7 meters from the ground showed a relative evaporation of 1.30.

A second set was run in a low upland with a thin belt of tall (21-25 meters) pines between it and the lake—thus protecting the atmometers in a measure from the prevailing wind. The ground was covered with vegetation, especially with blueberries interspersed with taller birch and aspen trees, thus giving conditions of an open aspen thicket somewhat protected from wind but rather fully exposed to the sun. The results from the different heights show: at 1.2 meters, 1.09; at 2.4 meters, 1.39; and at 3.7 meters, 1.37. The greater variation in gradations here not only made a difference in the ground rate, as was to be expected, but also blocked the exhibition of the usual increased rate of evaporation at 3.7 meters over that at 2.4 meters.

Set no. 3 was run in a low pine-land area which had been cleared and plowed for a fire-line some years previously. The vegetation above the open sod at the ground level was very open. The results at different heights show: at 0.6 meter, 1.15; at 2.1 meters, 1.25; and at 6.1 meters, 1.71. Here also the ground evaporation varied a little more than in the first set, but the results of increased height above the ground were very plainly visible.

Not far from this set were run a group of spherical atmometers in pairs of black and white spheres. At the ground the black showed 1.08 times the reading of the white; at 1.2 meters, the black showed 1.41 times the reading of the white at the same height. At 4.6 meters, the black showed 1.30 times the reading of the white at the same elevation. Comparing the white atmometers only at different elevations, that at 1.2 meters showed 1.28 times that at the ground, and at 4.6 meters, 1.65. Taking the black atmometers only, at 1.2 meters the relation was 1.67, while at 4.6 meters it was 1.97 times that at the ground.

#### BOG SERIES

During the studies of evaporation in bogs made during 1918, certain pairs and triplets of atmometers were located so that the results could apply on the present paper. The instrumentation in Bryant's Bog and at Gleason's Bog was carried on by Ruth Hurd West; that at Smith's Bog by Dorothy J. Cashen.

#### Bryant's Bog

Four series of atmometers were maintained within a space of perhaps 45 meters of each other. The bog itself is not over 120 meters across and located in a little kettle hole bordered on the west, south, and east by tree-covered ridge or forest and on the north by a narrow, low, tree-covered ridge ending in Douglas Lake. The general situation is one of considerable protection from the wind and of favorable conditions for warm, moist atmosphere, tending to cut down the amount of evaporation even from the most exposed places.

Set no. 1 was run in a clump of *Nemopanthus mucronata* about 23 meters from the open water in the south part of the bog. The vegetation was largely made up of high shrubs of *Nemopanthus*, medium-sized shrubs of *Vaccinium canadense*, and *Chamaedaphne calyculata*, with a few herbaceous species, especially *Carex trisperma*. An atmometer run at the crown of a *Nemopanthus* 1.9 meters above the ground gave a rate 1.41 times that in the center of a high clump of *Chamaedaphne* at 0.7 meter, while a second one at the level of the crown of *Chamaedaphne* 1.3 meters above the ground gave a rate 1.22 times that in the center of the clump at 0.7 meter.

A second set was run about 7.5 meters from the open water in *Chamaedaphne*, where there were a few plants of *Sarracenia purpurea*, a seedling of *Pinus strobus*, and a general ground cover of *Sphagnum*. The atmometer run in the crown of *Chamaedaphne* at a height of 0.7 meter gave a rate of 1.56 times that run close to the ground.

A third set was stationed in the *Chamaedaphne*, similarly to the second but 3 meters from the lake. It consisted of two spherical atmometers, one black and one white, run at 0.6 meter, the height of the crown of *Chamaedaphne*. The black atmometer showed 1.22 times the reading of the white atmometer.

A fourth set was run in a dense clump of *Nemopanthus* at the very edge of the bog. Other species in the immediate vicinity included lower shrubs of *Vaccinium canadense*, but most of the rest of the vegetation consisted of clumps of *Carex trisperma* and masses of *Sphagnum* in which was a little *Polytrichum*. The atmometer run in the crown of *Nemopanthus* 1.4 meters above the ground showed 2.03 times that at the ground.

### Gleason's Bog

Two sets of atmometers were run in Gleason's Bog, a small figure-8-shaped bog located in a large kettle-hole depression about three fourths kilometer east of Bryant's Bog. The water level of the bog is that of Douglas Lake. In years of high water, standing water is present in the bog; but in ordinary years, before the middle of the summer no standing water is present. The bog is almost uniformly vegetated with *Chamaedaphne*, with *Sphagnum* filling the spaces under and between the bushes, except towards the southern margin where there are a few higher shrubs that have not been wiped out by the frequent fires to which this kettle hole has been subjected.

The first set was run at the south margin of the bog in a clump of tall *Cornus alternifolia* with *Sphagnum* and *Chamaedaphne* quite abundant beneath it. An atmometer placed at the level of the crown of *Cornus*, 1.7 meters above the ground, gave 3.19 times that at the ground, while one placed in the crown of *Chamaedaphne* at 0.8 meter gave 1.96 times that at the ground.

A second set was run in the north-central part of the bog where a bush of *Nemopanthus* was beginning to shade out *Chamaedaphne*. The atmometer placed in the crown of *Nemopanthus* 1.6 meters in height gave 2.34 times that at the ground, while one placed in the crown of *Chamaedaphne* at 0.8 meter gave 1.30 times that at the ground.

### Smith's Bog

Smith's Bog is a well developed *Carex lasiocarpa* bog in which the floating mat has only recently become grounded. A small pool occurs in the center, surrounded for a considerable distance by the *Carex lasiocarpa* mat and giving place at the edges to various shrubs and at the extreme south to a little patch of lowland forest. Three sets of instruments were run here.

Set no. 1 was run in a patch of *Chamaedaphne* and *Salix pedicellaris* about 10 meters from the shore. Spherical atmometers were employed, a black and a white one at the crown of *Chamaedaphne* 0.9 meter above the ground. The white spherical atmometer showed 1.59 times that at the ground, while the black atmometer at 0.9 meter showed 1.25 times the reading of the white atmometer at the same height. This station was rather well exposed to the prevailing wind, and the fairly high evaporation is rather to be expected, even though the area was a bog with standing water.

A second set was run in a bush of *Chamaedaphne* that had invaded the

Carex mat rather close to the pond, close enough, as the atmometer readings show, to be affected by the proximity of moist air. An atmometer placed in the crown of the Chamaedaphne at 0.7 meter height showed 1.42 times that run at the ground.

A third set was run on the west side of the bog at the margin of *Salix pedicellaris* and Chamaedaphne, where the Salix was obviously beginning to push the Chamaedaphne rather hard. An atmometer run in the crown of the Salix at 1.1 meters showed 1.89 times that at the ground, and an atmometer run in the crown of Chamaedaphne at 0.7 meter showed 1.17 times that at the ground.

#### SEDGE POINT SERIES

Three sets of atmometers were run in the beach pools at Sedge Point during 1918 by Ruth Hurd West in connection with small trees of *Acer rubrum* occurring in the marsh vegetation which characterizes the second pool.

Set no. 1 was run in a small seedling of *Acer rubrum*, with small bushes of Salix and herbaceous plants such as *Typha latifolia* and *Carex lasiocarpa* also present. Here the atmometer run in the crown of the Acer at 0.9 meter gave 2.04 times that at the ground.

A second set in which spherical atmometers were employed was run in a young *Acer rubrum* surrounded by Typha and *Calamagrostis canadensis*. At a height of 0.7 meter, a white and a black sphere were run. The white sphere showed 1.44 times that at the ground, while the black sphere run at the same height showed 1.66 times the reading of the white sphere at the same height.

A third set was run in a medium-sized *Acer rubrum* seedling with a hardy *Salix lucida* adjoining and brown and straggling plants of *Scirpus validus*. An atmometer run in the crown of the Acer at 1.3 meters showed 2.00 times that at the ground, and one run a little lower down, at 0.8 meter, showed 1.39 times that at the ground.

#### SCIRPUS SERIES

During 1921 and 1922 several sets of atmometers were run in connection with some special work in Scirpus associations. Those run in 1921 were conducted in Deer Bay from July 2 to August 23, 1921, by Alice E. Keener, while in 1922 sets were run by Ruth Hurd West in Deer Bay, in a cove just west of Grapevine Point, at Maple Point, and at Maple Cove from June 22 to August 11. In each of these sets black and white spherical rain-correcting atmometers were used in pairs at the level of the tops of the culms of *Scirpus validus* (0.7-1.1 meters) and of *Scirpus americanus* (about 0.4 meter).

#### Deer Bay Sets

In Deer Bay the Scirpus associations are well developed in a crescent-shaped cove between Deer Point and Hook Point, fully exposed to southerly

winds only. On the north the forest-covered land, and farther back a forest-covered ridge, furnish a rather thorough protection from wind. The vegetation of these pools is mostly a very dense growth of *Scirpus americanus*, in a few parts of which, especially towards the western corner, fair-sized patches of *S. validus* are present. During 1921 two sets were run here—one in the western part of the largest beach pool where a large patch of *S. validus* adjoins an even larger patch of *S. americanus*. The results were as follows: At the level of the *S. validus* culms (1.1 meters) a black atmometer showed 1.69 times the reading of the white atmometer at the same level, while at the *S. americanus* level (0.4 meter) the black atmometer reading was 1.73 times that of the white. In the case of the white atmometers the higher elevation showed 1.27 times the reading of the lower, while in that of the black atmometers the higher elevation meant 1.24 times the rate at the lower elevation.

The second set was run in the middle of this largest beach pool very close to the middle of the cove at the tension line of a moderate-sized patch of *Scirpus validus* and a very extensive area of *S. americanus*. The results here, in this more exposed position, were as follows: For the white atmometer 1.25, while for the black atmometers the result in favor of higher elevation was 1.24. Comparing the black and white atmometers at the upper level, the black atmometer showed 1.58 times the reading of the white atmometer; at the lower level it showed 1.60.

During 1922 the set was run in exactly the same situation as was the former of the sets previously mentioned. In this year conditions were much less favorable for high evaporation. Comparing the black and white atmometers at the upper level, the black showed 1.25 with the white at unity, at the lower level the black showed 1.57. Comparing the white atmometers, the elevation made a difference of 1.34, while in the case of the black atmometers the difference was 1.07.

### Grapevine Point Set

The set run in the cove west of Grapevine Point was at the tension line between a moderately extensive growth of *Scirpus americanus* and a small patch of *S. validus* (*S. validus* here has suffered greatly from very severe ice work and is much less extensive than it was formerly. The same is also true of *S. americanus*, but conditions are much more favorable for its rapid extension). In this location there was marked protection from southerly winds in view of the tree-covered steep slope to heights of 6 or 7 meters. There was also a certain amount of protection from the northerly winds on account of the shelving beach extending out 100 or more meters; also, in breaking, the waves caused more spray and thus moister air around the atmometers than would otherwise have been the case. The results here showed, for the white atmometers at the higher level, an increased evaporation of 1.37 over the lower level, and for the black atmometers 1.40.



Considering the white and black atmometers at the higher level, the black showed 1.36 times the reading of the white; at the lower level the comparative reading of the black was 1.33.

### Maple Point Sets

At the west end of the lake two sets were run, one in the cove west of Maple Point and the other in the open lake just east of Maple Point.

The set east of Maple Point was located at the base of *Phragmites* Flat in a medium growth of *Scirpus americanus* at the edge of a moderate-sized although open patch of *S. validus*. In this situation fair protection was afforded from the prevailing westerly winds, but little or no protection from winds from any other direction. The winds from easterly directions had either the full sweep of the lake or, if from south of east, a sweep of over 1.5 kilometers. The results here showed for the black atmometers that the difference in favor of the upper level was 1.13; for the white atmometers, in favor of the upper level, 1.17. Comparing black and white atmometers at the upper level, the black atmometers showed 1.32 times the reading of the white at the same level; at the lower level the black showed 1.35 times the reading of the white at that level.

In the case of the cove, the atmometers were run at the edge of a sparse growth of *Scirpus validus* where it bordered the *Eleocharis* consociates of the *S. americanus* association. In this situation there was a certain amount of protection from most of the northerly winds because of the heavily vegetated, although low, land, and the location of the cove was such as to make wave action very slight. In this set the results were as follows: Comparing the black atmometers, there was a difference of 1.07 in favor of the higher level; in the case of the white atmometers the difference was 1.16 in favor of the higher level. At the higher level the black atmometer showed 1.31 times the reading of the white at the same level, and at the lower level the black showed 1.42 times the reading of the white at the same level.

### RESULTS AND INTERPRETATIONS

Plotting of the graphs in detail reveals the expected variation from period to period coincident with the varying meteorological conditions of the different days and years. With very few exceptions, the curves were similar and almost identical throughout, with the greater evaporation taking place from the blackened spheres and from the higher or more exposed positions. In view of the fact that such results have been tabulated and expressed in graph form in many papers by different authors in the past eight or nine years, and since the present results add to our knowledge only in other particulars, no attempt will be made here to express such variations. The interpretation of the results will deal exclusively with the results of the whole season's work, reducing for comparison the results of the different seasons to the rate per day in cubic centimeters of the evaporation for the season.

With the exception of the marsh series, each series was conducted within a year. Table I, however, permits a comparison of the evaporation of the different seasons.

TABLE I. *Rate of Evaporation from the Standard at Douglas Lake, Michigan, during Midsummer of Different Years*

Year	Seasonal Rate, cc. per Day	Year	Seasonal Rate, cc. per Day
1916.....	33.1	1920.....	31.4
1917.....	28.4	1921.....	29.2
1918.....	27.8	1922.....	23.1
1919.....	27.8		

It will be noted that the pine and aspen series in 1917 and the bog series in 1918 were run under very nearly equal evaporating conditions. In the case of the marsh series, however, two sets were run in a summer of high evaporation and four sets in one of low evaporation. This made a conspicuous difference in the rate of evaporation, as would be expected. The difference was much more pronounced in the case of black atmometers than in that of white instruments, as might also be expected.

### The Pine Series

There was considerable variation in the results or in the rates of evaporation in view of the diversity of the habitats and the separation from one another. The average increase of 6.06 cc./m./day was attained from records up to 4 meters. This includes one case very much below the average and one case very much above.

### The Aspen Series

In this series there was the closest approach to a uniform evaporation increase for the whole series; namely, 3.35 cc./m./day, even though the atmometers were not all in the immediate vicinity of one another. In the cases in which groups of atmometers were run close together, the results indicate a tendency for the evaporation to increase rapidly at first in the crowns of the trees and then still to continue to increase, but at a much slower rate. The modest levels at which the aspens grow in the region leaves the matter an open question as to whether the effect of elevation is continually carried on in the same manner. It is also undoubtedly true that an instrument in the open air above a tree would show greater evaporation than one in the crown of the tree, and the evaporation would be expected to diminish sharply in rate as the tree grew up to that height and brought the atmometer into its crown.

### The Bog Series

The outstanding feature of the bog series was the fact that here were obtained the highest figures for increases in evaporation in meters per day in all the cases in which the lower atmometer was maintained close to the

ground. As the increase was distinctly less in the cases in which the lower atmometer was higher up, the explanation is apparently quite simple. The evaporating conditions close to the ground in bogs are known to be quite low. This low rate is maintained by the proximity of standing water, the checking of the wind by bushes, and also by the fact that the bog itself is in a depression which makes air drainage more difficult. These conditions result in a low rate close to the ground. This, in comparison with an ordinary rate higher in the air, would show a great increase in rate per meter.

### The Marsh Series

The marsh series gave the same general results as the bog series; namely, a high increase in meters per day, but not so great an increase as in the bog set. The proximity of Douglas Lake undoubtedly had a blanketing effect on the evaporation, especially from the atmometers exposed to winds from over the lake, and doubtless even more so on the evaporation from the atmometers nearer to the ground. Even under these conditions, however, the increase was 7.83 cc./m./day for white atmometers and 8.99 cc./m./day for black atmometers. The maximum height for any of the instruments in this series was, however, only 1.1 meters.

These figures for a rather openly disposed vegetation in midsummer are of course higher than those obtained by Sherff<sup>6</sup> in autumn in denser vegetation, namely, 1.59 cc./m./day in comparing 1.07 meters with 0.25 meter in *Phragmites* in Skokie Marsh, or 1.77 when comparing an atmometer at 1.98 meters in the top of *Phragmites* with one at 0.25 meter. In the case of *Typha* the figures were 1.46 cc./m./day in comparing atmometers at 1.07 meters and 0.25 meter, and 3.27 when comparing one at 1.75 meters (the top of *Typha*—browning and falling over towards the close of the experiment) with one at 0.25 meter.

Looking over the results as a whole, it is plainly evident that there is always an increase in the rate of evaporation as one increases the height of the instruments above the ground. The wide range of values, however, also clearly shows that the local factors are of great importance in determining the actual magnitude of increase. Likewise, the region and the time of year need consideration. In the present work the increase in height amounted to from 0.2 meter to 5.8 meters. The atmometers were maintained at the top of the crown in order to evaluate the conditions that the plants in question were meeting as they grew higher from the ground levels at which they started. The climatic variations in different years, as shown in table 1 (the evaporation from the standard), account for a wide variation in values for any given spot. Aside from this fact, however, the greatest rates of increase were in the bog sets because the ground rate was there so distinctly low. Next came the marsh series and last the upland tree series, although this last series had the highest actual rate of evaporation.

<sup>6</sup> Sherff, E. E. *Loc. cit.*

TABLE 2. *Evaporation Rates in the Vicinity of Douglas Lake, Michigan, for 40-52 Days inclusive of the Period of Maximum Evaporation*

	Year	Height of Atmometer in Meters		Evaporation, cc. per Day		Rate of Increase upward, cc. per Meter per Day
		Lower	Upper	Lower	Upper	
White Atmometers						
Seedling and Small Pine Trees ( <i>Pinus strobus</i> and <i>Pinus resinosa</i> )						
Set 1.....	1917	0.3	4.0	22.76	26.82	1.10
Set 2.....		0.3	1.8	16.95	17.33	0.25
Set 3.....		0.3	1.5	10.40	16.81	5.34
Set 4.....		0.3	0.5	11.71	15.21	17.55
Aspen Trees ( <i>Populus grandidentata</i> and <i>Populus tremuloides</i> )						
Set 1.....	1917	0.3	0.9	17.88	20.78	4.83
		0.3	1.8	17.62	24.56	4.63
		0.3	2.7	15.54	20.22	1.95
		0.3	3.7	17.66	26.13	2.49
Set 2.....		0.3	1.2	14.33	15.55	1.36
		0.3	2.4	11.94	16.58	2.21
		0.3	3.7	13.18	18.11	1.45
Set 3.....		0.3	0.6	14.80	17.04	7.47
		0.3	2.1	16.12	20.09	2.21
		0.3	6.1	14.18	24.31	1.75
Set 4.....		0.3	1.2	21.25	27.26	6.68
		0.3	4.6	21.25	34.97	3.19
Bog Shrubs ( <i>Chamaedaphne calyculata</i> , <i>Nemopanthus mucronata</i> , <i>Salix pedicellaris</i> )						
Bryant's 1.....	1918	0.7	1.3	12.97	15.88	4.85
		0.7	1.9	12.97	18.32	4.46
Bryant's 2.....		0.3	0.7	9.46	14.76	13.25
Bryant's 4.....		0.3	1.4	10.90	22.14	10.22
Gleason's 1.....		0.4	0.8	8.15	15.94	19.48
		0.4	1.7	8.15	26.00	13.73
Gleason's 2.....		0.4	0.8	10.54	13.73	7.98
		0.4	1.6	10.54	24.68	11.78
Smith's 1.....		0.4	0.9	16.86	26.84	19.96
Smith's 2.....		0.4	0.7	12.40	17.64	17.47
Smith's 3.....		0.5	0.7	10.13	11.89	8.80
		0.5	1.1	10.13	19.19	15.10
Bog Trees ( <i>Acer rubrum</i> )						
Sedge Point 1.....		0.3	0.9	7.84	15.98	13.57
Sedge Point 2.....		0.3	0.7	10.92	15.69	11.93
Sedge Point 3.....		0.3	0.8	9.36	13.05	7.38
		0.3	1.3	9.36	18.73	9.37
Marsh Herbs ( <i>Scirpus validus</i> and <i>Scirpus americanus</i> )						
Deer Bay, Set E....	1921	0.4	1.1	17.23	21.62	6.27
Deer Bay, Set W....		0.4	1.1	15.54	19.70	5.94
Deer Bay.....	1922	0.4	1.1	11.40	15.28	5.54
Grapevine Point...		0.4	0.8	14.39	19.72	13.33
Maple Point.....		0.4	0.7	17.84	20.82	9.93
Maple Cove.....		0.4	0.9	18.20	21.19	5.98
Black Atmometers						
Aspen Set 4.....	1917	0.3	1.2	23.02	38.40	17.10
		0.3	4.6	23.02	45.34	5.19
Deer Bay, Set E....	1921	0.4	1.1	27.63	34.24	9.44
Deer Bay, Set W....		0.4	1.1	26.88	33.30	9.17
Deer Bay.....	1922	0.4	1.1	17.95	19.14	1.70
Grapevine Point...		0.4	0.8	19.11	26.76	19.13
Maple Point.....		0.4	0.7	24.14	27.39	10.83
Maple Cove.....		0.4	0.9	25.93	27.75	3.64

TABLE 3. *Summary of the Rates of Increase in Evaporation from Different Levels in Different Types of Vegetation (White Atmometers unless Otherwise Stated)*

Height of Lower Atmometer in Meters	No. of Sets	Vegetation	Rate of Evaporation Increase in cc./m./Day
0.3	4	Pine crowns	6.06
0.3	12	Aspen crowns	3.35
0.3	2	Black atmometers in aspen crowns	11.10
0.3	2	Bryant's Bog	11.74
0.3	4	Sedge Point	10.56
0.4	4	Gleason's Bog	13.24
0.4	2	Smith's Bog	18.72
0.4	6	Scirpus	7.83
0.4	6	Black atmometers in Scirpus	8.99
0.5	2	Smith's Bog	11.95
0.7	2	Bryant's Bog	4.66

In the eight sets which permit comparison of black atmometers at different levels, all show decisively increased evaporation at the higher level. Six of the eight sets yielded higher rates of increase with the black atmometers than were obtained in the corresponding series with white atmometers. In both of the cases in which the black atmometers failed to show so great an increase as the white, the atmometers were in coves protected more or less against wind and to which the sun did not have access for so long a time.

#### SUMMARY

1. In the Douglas Lake region in Cheboygan County, Michigan, studies have been carried on for several years which enable a comparison of the rates of evaporation in the crowns of different plants at different levels above the ground.

2. An increase in evaporation was uniformly shown, even if the atmometer at the higher level was only 0.2 meter above the lower.

3. It appears that, in the crowns of the plants utilized, the increase is rapid at first but decreases with increase in height.

4. The greatest rates of increase appear in the bog series where, however, the normally much lower evaporation at the ground level was responsible for this anomaly. The actual rate of evaporation is dependent upon the local conditions.

5. A plant meets conditions of increasing severity as it grows upwards from the ground. In this region and in this series of experiments, this change in conditions has meant, under the conditions of experimentation, an increase of 6.06 cc./m./day (to 4 meters) in pine groves; 3.55 cc./m./day (to 6.1 meters) in aspen groves; 13.78 cc./m./day (to 1.9 meters) in bogs; 10.56 cc./m./day (to 1.3 meters) in a bog-swamp; and 7.83 cc./m./day (to 1.1 meters) in marshes—all with white atmometers. With black atmometers the increases were 11.1 cc./m./day (to 4.6 meters) in aspen groves, and 8.99 cc./m./day (to 1.1 meters) in marshes.

## CHROMOSOME MORPHOLOGY IN FRITILLARIA, ALSTROEMERIA, SILPHIUM, AND OTHER GENERA

WM. RANDOLPH TAYLOR

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As a result of studies on somatic chromosomes in plants, there have been demonstrated at most three stable features which may characterize the forms exhibited. The presence of these elements in the various possible space relationships between each other and the ends of the chromosome gives all that may be recognized as characteristic beyond the mere size. Other features may of course be found in time.

Most careful study of the more minute chromosomes fails to disclose longitudinal differentiation other than that involved in the shape of the ends of the chromosomes, in so far as they may be rounded, truncate, or gradually tapering. The writer has studied in particular several species of *Acer* (22) and also *Aechmea* and *Cleome* (26), *Ricinus*, *Bryophyllum*, *Cucurbita*, *Populus*, and *Salix*, as well as members of other genera on which he is not ready to report. With chromosomes of a slightly larger size it is possible to distinguish in the anaphases a distinct flexure at the region of the spindle-fiber attachment, and in metaphases or anaphases or in both it is often clear that the chromosome is more slender at this region than farther down along the shaft, or along the two arms if it is "V"-shaped. This is the first of the three features which determine specific chromosome configuration. Some plants contain chromosomes of such a wide range in size that part of the complex shows no indication of a special fiber-attachment region, while another part may exhibit such a zone; of these, *Yucca filamentosa* is a case in point. Generally the chromosomes are more nearly of the same order of magnitude and show more or less clearly the attachment region. Examples of such a condition include *Coix Lacryma-Jobi*, *Fagopyrum esculentum*, and *Eichhornia speciosa* (26). Such as these are on the border line where the small size of the elements requires a considerable refinement of technique in order that the small though characteristic irregularities of outline may not be obliterated. If one studies plants containing larger chromosomes it becomes possible to find this attachment zone with greater ease. The writer has studied members of several genera with moderate to large chromosomes in which it is easily seen, and which may be listed roughly in order of the size of the elements: *Velthemia* and *Cyrtanthus* (24), *Sagittaria* (26), *Haworthia*, *Aloë*, and *Gasteria* (25), *Alstroemeria* and *Fritillaria*. Just what causes the constriction at the region of fiber-attachment is not known. Newton, working on *Galtonia*, recognized these constrictions in the

prophases of the somatic mitoses, and the writer has studied them in the second maturation-division prophases of *Gasteria* and *Veltheimia*. As the nuclear membrane remains intact until long after the constrictions have become established, it can not be considered that they are caused by a tightening of the fiber about a region to which it is applying a tractive force. On the contrary, the region to which the fibers are to be attached is determined before the fibers can at all become oriented with respect to the chromosomes. Further, it is hardly established that the fibers do actually by contraction or otherwise drag the chromosomes toward the spindle poles, although certainly the picture presented by suitably fixed preparations would confirm such an impression. It has been represented to the writer that those of his preparations which show the fiber-attachment constrictions, satellites, etc., in the most superior fashion do not show much trace of spindle fibers, especially in the microspore-maturation smears. This is to be considered as due to the rapid and very uniform precipitation of the cytoplasm, especially by the action of the osmic acid, which is very effective under these conditions. Such treatment is necessary to reduce the longitudinal contraction of the chromosomes, which would obliterate the features under study, and to reduce the clumping of the chromosomes, which would render observation difficult, but with respect to the cytoplasm is to be considered as producing an over-fixation because the several colloids present have been precipitated with too slight density-differences for our staining methods to become effective in differentiating between them. Furthermore, the stain used, Heidenhain's iron-alum haematoxylin, is not suitable for a study of the spindle. But when a highly selective stain of safranin-gentian violet is secured on root-tip material in which a rapid but more gentle precipitation of the cytoplasm has taken place, the attachment constrictions, satellites, etc., can be distinguished at least tolerably well, and the fibrous nature of the spindle with the orientation of heavier fibers at the constriction zones can be demonstrated.

To what degree the fiber-attachment constrictions are general in larger chromosomes it is too early to state. The writer is inclined to the opinion that an actual constriction zone or related structural differentiation is always present and can be demonstrated by suitable means. It might be considered as obvious that an exception to this statement should be made in cases of terminal fiber-attachment. But the writer has failed to find a case among any of the medium or large chromosome types studied in which a chromosome had a truly terminal fiber-attachment in the second maturation division, in the first pollen-grain mitosis, or in somatic mitoses. There is always at least a sub-spherical knob about the base of which attachment seems to be effected. This is true even of those chromosomes which bear proximal satellites and in which the attachment constrictions are particularly hard to demonstrate. The small chromosome types can not be cited because the difficulties in demonstration of the conditions present

are too great to make the evidence conclusive, and the first maturation division, while it does show constancy of spindle-fiber attachment, has not been shown to possess either attachment constrictions or satellites.

The second of the three notable features of chromosome form consists in the partial isolation of a distal portion of the chromosome shaft. The separation is usually not great and is bridged by a strand, probably of linin substance, which may at times appear to be double (25) and is usually slender, although cases have been reported of light bands across an unconstricted shaft which may be a somewhat different manifestation of the same fundamental feature. The relative size of the body thus isolated is fairly constant for a given plant, and varies from a sphere with diameter less than that of the chromosome proper (*Gasteria*) to a body almost as long as the rest of the arm of the chromosome to which it is attached (*Vicia faba*), and even proportionately longer bodies may in time be found to be attached to that proximal part of the chromosome which connects with the spindle fiber. This type of body has been studied by the writer in *Gasteria*, *Fritillaria*, *Cyrtanthus*, and *Vicia*. In *Gasteria*, where the maturation divisions were studied, it did not seem to be possible to distinguish in these this "satellite" body. There is no clear demarcation between this kind of satellite and that for which the name was originally adopted, and which constitutes the third of the features of chromosome configuration to be considered.

S. Navashin in 1912 discovered in *Galtonia candicans* that two chromosomes each carried on the proximal end a tiny sphere attached by a slender thread. Subsequently a few other workers have reported similar structures in other plants. The body is generally a small one and the cord of attachment quite long, so that usually one is able easily to class this type of structure as distinct from that considered above. But *Crepis setosa* has really quite a large proximal satellite while *Aloë* and *Gasteria* have quite small distal ones, and in *Fritillaria* the separation of the distal body may be quite extended while in *Haworthia* the separation of the proximal one may be normally slight. The actual amount of separation is in all these cases apparently reduced by slow fixation or shrinkage of the protoplast. So, while the facies of the proximal satellites is quite distinct from that of the distal ones, a really scientific distinction may not be possible. S. Navashin (12, 13), in distinguishing two races of *Galtonia*, one having the proximal satellites of equal size and the other having them unequal, has assigned great importance to these elements in relating the two classes to fundamental differences in racial vigor. It is indeed possible that there is a functional difference between the class of proximal satellites and that of the distal elements, but beyond the studies of Navashin there has been no correlation shown between either class of satellite and morphological or physiological features. In the cases studied by the writer minor differences between satellites in a given cell have been observed, as well as between the corre-



sponding pairs in different roots, but the exact comparison of individual plants necessary to a verification and extension of Navashin's observations or to a contradiction of them has not been made.

It has been suggested that by means of the separation of the constricted limbs of chromosomes a permanent increase in the chromosome number in the cell and its descendants may be effected. In cases in plants in which increase in chromosome number has been effected by mutation, by hybridization, or by narcotization, and in which the exact morphology of the elements is known, it has been found that the extra element or elements are the exact duplicates of chromosome types already present in the complexes from which the new cell type had been derived, and that they merely result from irregularities in separation at some previous anaphase. On theoretical grounds this explanation of the origin of supernumerary chromosomes offers difficulties, for the new fragment-element would have to assume a fiber-attachment constriction, and since this is a stable hereditary character of the chromosome it might involve complicated changes in the prophases long preceding the anaphases in which an irregularity of distribution would cause the extra chromosome piece to become added to a normal assortment. Positive evidence of fracture must involve, beside an increase in number, a demonstration that the two supposed halves correspond in more exact and fixed anatomical characters than mere size, and must involve the demonstration of a new fiber-attachment in its relation to the morphological characters already present.

The effects of the action of fixing fluids of various types upon the prominence of these various features are important in relation to the interpretation of the results of future workers in this field. Such a study has progressed to an advanced point on *Allium* and *Gasteria* and will be reported upon in detail later. It may be interesting to record here, however, that the satellites in the onion have been found after treatment with most of the standard mixtures except perhaps Carnoy's and Farmer's fluids. In general, the chrom-osmo-acetic mixtures with maltose have afforded the most uniformly dependable results, and all the original data given in this paper have been derived from material fixed in this way. Narcotics also are reported to have a notable effect upon the constrictions, emphasizing greatly their prominence. This work has been reviewed in an earlier paper (23).

The foregoing exposition of the present state of this subject, although based primarily on personal experience, may serve in place of an extended review of the literature, which has in fact been outlined in previous articles on this topic (23-26). In fact, while several writers have given casual observations which may be construed as bearing on this problem, the number of writers who have given the matter direct attention and who have produced real and valuable evidence is not great. In addition to the previously recorded work we have recent papers by Mrs. Sorokine (18) on

*Ranunculus* and by Belling (2, 3) on *Hyacinthus*, and some data on *Crepis* which will be taken up later. Mrs. Sorokine worked with *Ranunculus acris* L., using material obtained in Russia. The species is normally diploid, but triploid gynodimorphic plants were found as well as intermediate ones. In the diploid plant two chromosomes of the group of twelve bear satellites, which are unequal in size and in this respect comparable to those described for *Galtonia* by Navashin. If the triploid plants were formed by the fertilization of a simple diploid gamete, with the usual chromosome complement simply doubled, by a haploid gamete, the result would be a triploid embryo and plant with three heterochromosomes each bearing satellites. But in the case as reported only two of the eighteen chromosomes bear satellites, and one of these has two satellites side by side. If the triploid plants studied did not have so simple a history, and were derived by crossing back and forth between a number of 12-, 18-, and 24- chromosome parents in such fashion as to cause various recombinations of the members of the complex, it can be understood how an 18-chromosome type might be produced in which the third homologue of the two heterochromosomes might have been replaced by a non-homologous element. Such a history might account for the lack of the third heterochromosome, as indeed it might be accounted for by considering that it had been overlooked, were it not for the statement that there are two satellites present on one of the heterochromosomes instead of one. These two bodies certainly suggest similar appearances seen by the writer in *Allium* and other plants, where the satellite had quite clearly divided in preparation for the metaphase-anaphase separation. In view of the fact that Mrs. Sorokine has not given any figures of this paired condition in the anaphases, the question may be raised as to whether in *Ranunculus* the pair as seen in the metaphases may not really simply have undergone a somewhat premature split. The prophase figures are not altogether convincing. There is finally the scant possibility that a mutation involved the failure of one of the satellites to separate.

Belling (2) has compared the chromosome complexes in the pollen grains of diploid and of triploid *Hyacinthus orientalis*. In the horticultural form "Yellowhammer" he reports four long "V"s with a constriction at the center; two medium-sized elements had unequal arms ("J"s) also with a constriction at the bend, and two small elements had a rather closely subterminal constriction. In the form "Lady Derby" the number of chromosomes varied from 8 to 14, and in the one cell which had 8 the assortment was the same as in "Yellowhammer." In the other cases various assortments of the types of chromosomes were observed, and these when tabulated showed a tolerably close approximation to a chance distribution of the elements present in the diploid complex. In a later paper (3) he confirms the constitution of the diploid complex and indicates that in the prophases of the first maturation division of the triploids trivalent groups of elements rather irregularly arranged are produced which correspond to the

bivalent groups organized in diploid plants. He clearly recognizes the fiber-attachment constrictions at the bends of the chromosomes.

#### FRITILLARIA IMPERIALIS L.

Gasteria offers the most easily interpreted cell complex of the monocotyledonous plants which the writer has studied. For this reason it is a highly desirable type, but the slowness with which roots form from cuttings and the slight difficulty in fixation caused by the mucilage render it somewhat less convenient than *Fritillaria*. In the latter form, the large number of root tips made available between the soil and the pot by turning out rapidly rooting bulbs furnishes an excellent source of easily fixed roots. The chromosomes are larger and so much greater in number that it is hardly possible to analyze directly the entire complex, or to secure intelligible metaphase polar views, but the anaphase side views are superb.

Some of the more notable features of the chromosome organization have been already reported for the genus. Strasburger (19, figs. 24, 25) figures for *Fritillaria persica* a first-maturation equatorial plate of 12 chromosomes, of which one pair appears to be longer than the others and bent to a "V" shape. He also (20) refers to *F. imperialis*, and figures mostly "V"-shaped chromosomes, with some "J"-shaped ones, in the endosperm. Guignard (5, 6), primarily concerned with fertilization, mentions and figures *F. meleagris*, but not in a fashion to aid in the present problem. Belajeff (1) also used this plant, studying the maturation divisions. It would appear that he had a rather clear understanding of the tetrad derived from the terminal-attachment type of chromosome in the first maturation metaphases, and possibly also of that derived from the median-attachment type. Van Wisselingh (27) used *F. imperialis* in studies on the nuclear reticulum. He observed conditions remarkably similar to those pictured in figure 19 of the present paper, but in view of his technique the significance of his results is questionable. Sax (17) was concerned with the fertilization phenomena, and gives no details of chromosome morphology in *F. pudica*. But Sakamura (16), who studied *F. camtschadensis*, recognized clearly the attachment-constriction regions and certainly one type, probably two types, of chromosomes with free constrictions on the long shaft. The chromosomes he figures in text figure 3, page 33 of his paper, correspond to my figures 2, 3, 5, and possibly 4. He does not seem to have completely analyzed the complex. He reports S. Navashin as having recognized the differences between the configurations of the chromosomes of *F. tenella* caused by differences in position of fiber-attachment, but the writer has not succeeded in locating this reference.

In *F. imperialis*, used for the present study, five types of chromosomes seem to be present. The first (text fig. 1) is a long shaft with a rather large elliptical lobe beyond the attachment zone. There are probably seven pairs of this shape, but these show indications of some differentiation among

themselves on the score of length. This it was impossible to analyze because of the involved character of the anaphase groups. The second type (text fig. 2) is a "V"-shaped element with the attachment zone somewhat aside from the center; there is usually not more than a 25-percent difference in length between the arms. Of this type there appear to be two pairs. Third, one finds (text fig. 3) a rod-shaped type with a small sub-spherical



TEXT FIGS. 1-19. *Fritillaria imperialis*, root-tip mitoses. FIGS. 1-5. Examples of five types in late anaphase. FIG. 6. Partial anaphase group, satellite attachments marked by arrows. FIGS. 7-14, 18, 19. Sister chromosomes in early anaphase. FIGS. 15-17. Late anaphase chromosomes.  $\times 2000$ .

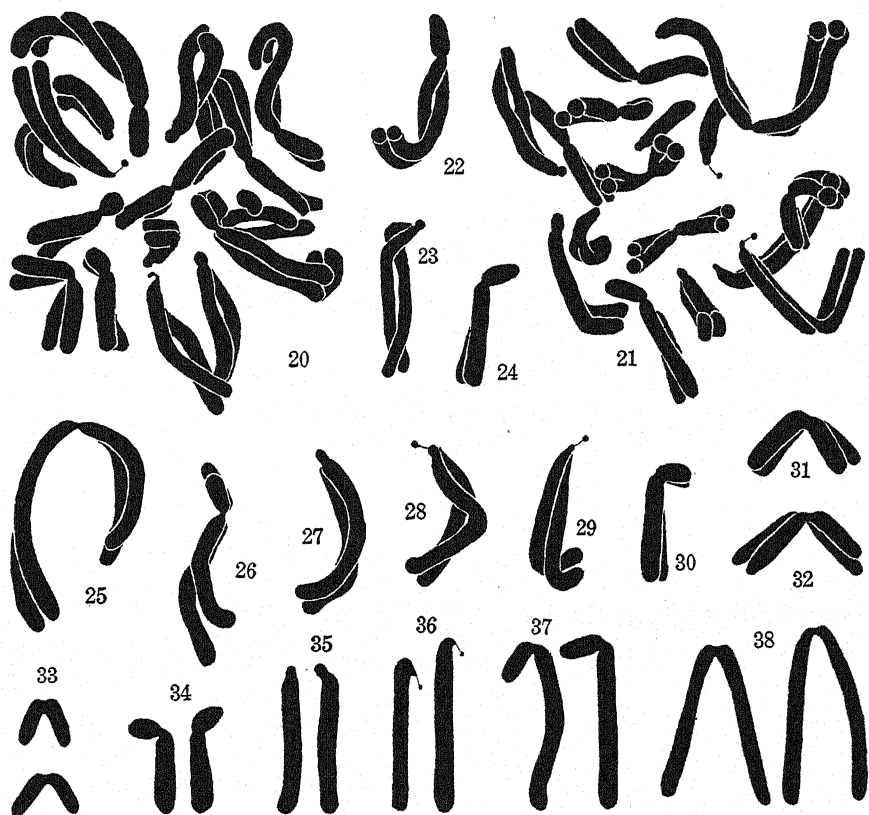
head end, represented probably by but one pair. There are finally two pairs of rod-shaped elements which have constrictions in the shaft. The first of these (text fig. 4) has the partially detached region amounting to at least one third of the whole length of the shaft. In the other (text fig. 5) this region comprises only about one sixth of the length of the shaft. These two pairs show differences also at the spindle-fiber-attachment region, for the first has a decidedly larger proximal lobe, its length twice its breadth, while that of the chromosome shown in text figure 5 has a very small, round head. Also the degree of detachment of the constricted body is characteristically different. The condition shown in text figure 15 is much more usual for the second type than is that shown in figure 5, while for the first type it is really quite rarely that the constricted body is in contact with the main shaft at all, though this condition is shown in text figure 17. In the large anaphase group of text figure 6 the usual condition is pictured with the first type of element, of which both anaphase sister pairs show, clearly different in degree of constriction from the second type which is represented by one anaphase sister pair on the left-hand side of the group. In this figure, cut or broken ends of chromosomes show in several places as clean, right-angled ends, but where one of the long rod-shaped elements crosses directly above one of the constricted elements on the lower left it has been indicated merely in dotted outline to permit the drawing of the more important feature clearly. There is but one pair of each of the last two described types, and probably but one of that represented in figure 3 also.

*Fritillaria* is a splendid plant in which to demonstrate the manner of drawing apart of the split somatic-metaphase chromosomes. The prophase spireme does not seem particularly suited for a study of the early stages because of the irregularly granular condition of the resting nucleus, but in later stages the large elements are especially favorable for study. As the early anaphase pairs begin to draw apart, the region of fiber-attachment first separates and the rest of the chromosomes progressively uncoil and follow after. The distal ends never seem to initiate the process or to move apart in a somewhat independent fashion, as they do in the maturation divisions of *Gasteria* and other plants. These points are well shown in text figures 7-14, 18, and 19, which cover all of the five recognized classes.

#### ALSTROEMERIA BRAZILIENSIS SPRENG.

*Alstroemeria* is more easily studied in the metaphases of somatic cells than *Fritillaria imperialis* because of the lower chromosome number. Strasburger (19) worked with *A. chilensis* Lood. and observed  $n = 8$  chromosomes in microspore-development. His figure shows little more than a slight size difference between the chromosomes. Guignard (4, 5, 6) studied *A. pelegriana* and *A. psittacina*. His figures of the former in the maturation divisions permit of interpretation as showing both median and terminal fiber-attachment. For the latter he shows "J"-shaped chromosomes and reports  $n = 8$  for both species.

The material studied by the writer was cultivated under the name of *A. braziliensis* and fitted the description of that species tolerably well. The root tips showed  $2n = 16$  chromosomes of quite different sizes, and the elements fall into six classes. The largest chromosome pair has the fiber-attachment constriction near its centre (text figures 25, 38). The second pair has a



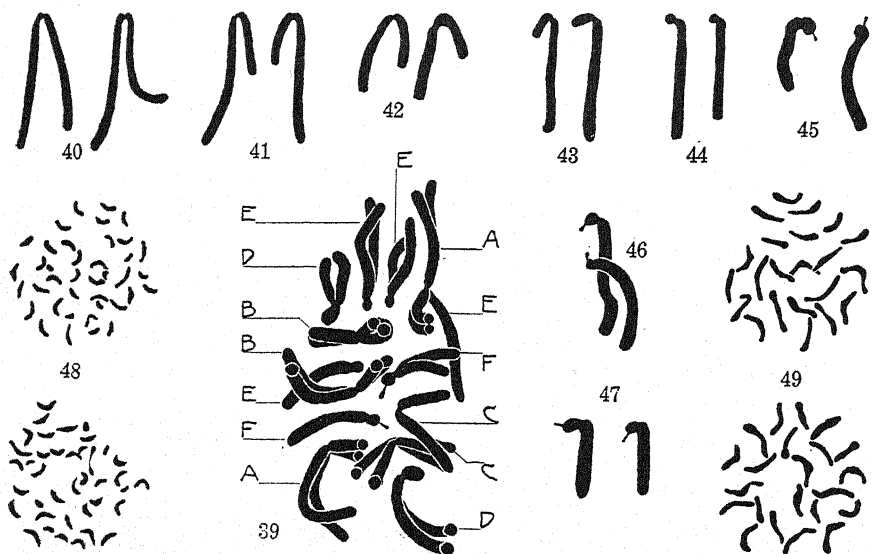
TEXT FIGS. 20-38. *Alstroemeria braziliensis*, root-tip mitoses. FIGS. 20, 21. Polar views, equatorial plates. FIGS. 22-32. Split metaphase chromosomes of the six types. FIGS. 33-38. Late anaphase examples of the six types.  $\times 2000$ .

long shaft and a well developed proximal lobe about 2-3 times as long as broad (text figs. 22, 26, 37). The third type is represented by three pairs of rods with short, rounded attachment ends (text figs. 23, 27, 35). This genus, like others that have been described, shows a pair of heterochromosomes, or satellite-bearing elements. These are not at all as easily demonstrated as in some cases, such as *Crepis*, but are fairly distinct in the metaphases (text figs. 28, 29, 36). In the anaphases it becomes almost impossible to trace them, both because of their small size and because of the grouping of the chromosomes. Fifth, there is a pair of small elements with proximal

lobes about twice as long as broad (text figs. 24, 30, 34), and sixth a pair of small elements with sub-median attachment constrictions (text figs. 31-33). This plant is not a particularly good one for study, for, although occasional equatorial plates are well spread out and can be studied effectively, in general they are very much crowded because of the large size of the chromosomes, the major portion of which are quite long, and because of the small size of the cells.

#### SILPHIUM PERFOLIATUM L.

This genus has been studied cytologically by Merrell (10) and by Land (7). Five species, *S. trifoliatum*, *S. terebinthinaceum*, *S. laciniatum*, *S. integrifolium*, and *S. perfoliatum*, were studied, primarily with respect to embryo-sac development. Merrell gives the count as  $n = 8$ . His figure 54, which is cited as evidence of the number, represents an atrociously clumped first-metaphase polar view, and can hardly be considered good evidence for any count. Land expresses himself as unable to make an accurate count, but, depending on Merrell, reports that 16 appears to be the chromosome number in the embryo and 24 in the endosperm. The writer has used material of *S. perfoliatum* from seedling root tips and from abundant root tips produced by rhizomes lifted in early spring and started into growth in sand and in sphagnum. The chromosome number certainly is  $2n = 14$ , and six classes of chromosomes are present. The largest has a median or submedian fiber-attachment (text figs. 40, 39A), and is represented by a single pair,



TEXT FIGS. 39-47. *Silphium perfoliatum*, root-tip mitoses. FIG. 39. Polar view, equatorial plate, the six classes of chromosomes marked A to F, corresponding to figures 40 to 45, which show late anaphase examples of the six classes. FIGS. 46, 47. Anaphase chromosomes of class F. FIG. 48. Two polar views of equatorial plates, *Bryophyllum calycinum*. FIG. 49. Two polar views, equatorial plates, *Freesia refracta*.  $\times 2000$ .

as is the second type, which has one arm less than half the length of the other (text figs. 41, 39B). The third pair has the fiber-attachment submedian, so that one arm is one third shorter than the other, and the whole chromosome is much smaller than that of the first type (text figs. 42, 39C). The fourth type is rod-shaped, with a proximal lobe about twice as long as broad (text figs. 43, 39D). The fifth is also rod-shaped, but with a rounded fiber-attachment end (text figs. 44, 39E), and is represented by two pairs. The sixth type is often indistinguishable from the fifth, but in favorable places there can be seen short, rodlike appendages to the proximal ends which, although perhaps hardly to be dignified with the title of satellites, probably represent the same type of structure (text figs. 45-47, 39F). They are much more easily seen in the metaphases than in the anaphases, and show fairly well in the polar view of an equatorial plate given in figure 39, in which the class to which each chromosome belongs is indicated by the appropriate letter.

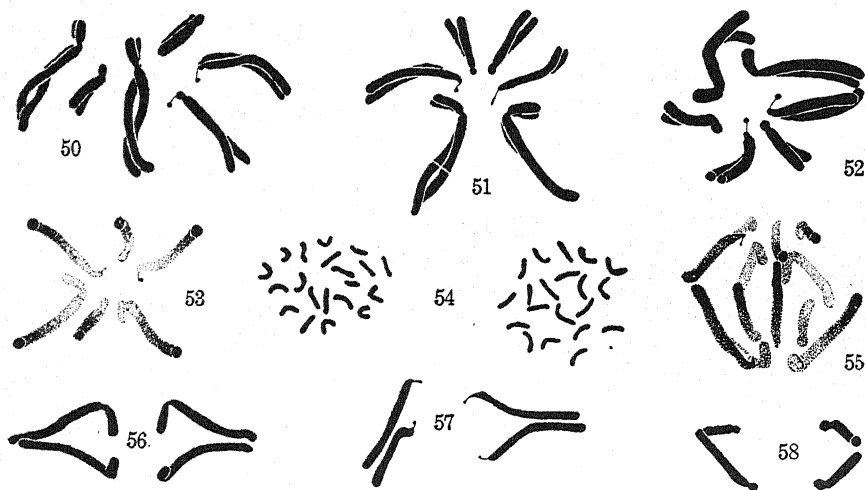
#### CREPIS CAPILLARIS (L.) WALLR.

In an earlier paper the writer has noted certain features of *C. setosa* Hall and *C. capillaris* (L.) Wallr. Additional very good material has suggested the presentation of some supplementary remarks on *C. capillaris*. In addition to the literature already cited, attention should be called to two papers by Rosenberg (14, 15). In the earlier paper he seems to have recognized the flexures as fairly constant, especially in *C. tectorum*, but failed to recognize attachment constrictions or satellites. In the later paper he does recognize the satellites on the mid-chromosome type and also describes a cross-segmentation which corresponds to the attachment constrictions. Litardiè (8) mentions several plants as having exhibited permanency of fiber-attachment, especially *C. capillaris* (*C. virens*) in which he recognizes one largest pair as having a low subterminal and the others as having terminal fiber-attachments. Miss Mann (9) in a very recent paper gives further data on the chromosome numbers in *Crepis*. This paper affords quite an inadequate idea as to the individual characters of the chromosomes in the different complexes. The author recognizes satellites as present on a pair of chromosomes of *C. setosa*, not only in the pure species but in hybrids of which it is a parent, while she misses the smaller ones in *C. capillaris*. She takes up favorably the suggestion of cross division as a possible method of increase in chromosome numbers, suggesting for instance that the two shortest chromosomes of *C. neglecta* might have been derived by cross division of the chromosome of middle length in *C. capillaris*; but, being probably unaware of the presence of the satellite on this chromosome in *C. capillaris*, she does not offer any solution as to its fate in this process. The chrom-acetic technique employed is of course quite too crude for the study of constrictions or of any but the largest satellites, as these structures show after fixation with this fluid only in sporadic instances.

In root-tip cells, *C. capillaris* shows six chromosomes, in pairs of three



sizes (text figs. 50-52). Each of the largest chromosomes has a proximal arm two or three times as long as broad beyond the attachment constriction (text fig. 56). The middle-sized pair have rounded head ends beyond the constrictions and distinct round satellites attached to these by cords in



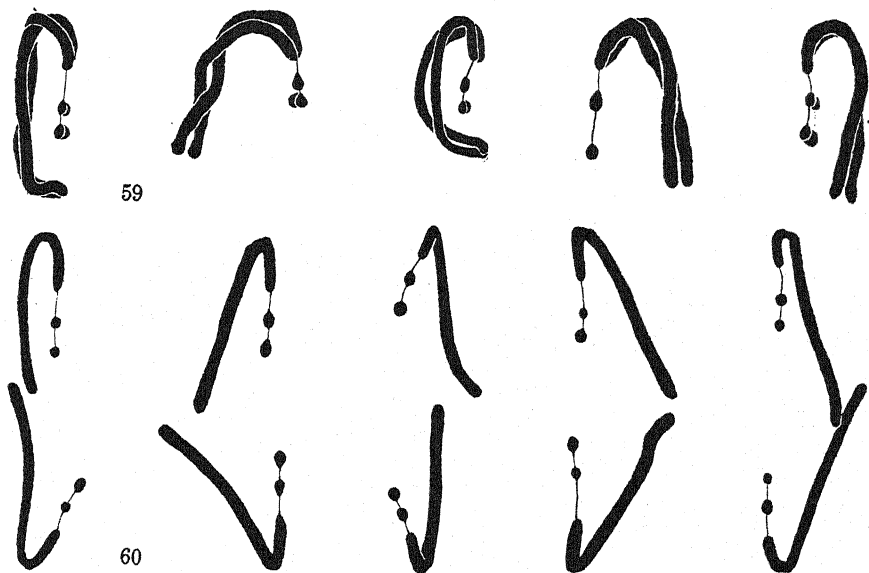
TEXT FIGS. 50-53, 55-58. *Crepis capillaris*, root-tip mitoses. FIGS. 50-52. Polar views, equatorial plates. FIG. 53. Polar view, one anaphase group. FIG. 55. Side view, complete anaphase. FIGS. 56-58. Three early anaphase homologous pairs, all from the same cell. FIG. 54. Two equatorial plates, root-tip mitoses, *Ricinus communis*.  $\times 2000$ .

length about two or three times their diameter (text fig. 57). In the earlier note the writer stated incorrectly that these were attached to the smaller pair. This error occurred in transcribing the notes, for the figure is correct. The smallest pair of chromosomes are short rods with rounded ends (text fig. 58). As the chromosomes pass into anaphase the flexures at the attachment constrictions become clearly established, but the satellites decrease in size as nuclear division progresses (text figs. 53, 55).

#### ALLIUM CEPA L.

The writer has continued a study of mitosis in the onion in connection with a most interesting departure from the condition which he previously described (24). There appeared in a large fixation of onion roots made in the winter of 1923-24 a few which showed an entirely different satellite relation from that which had become familiar. Most of the chromosomes were long "V"s as before, but in place of a pair of "J"s bearing each a small satellite on the short arm there showed one splendidly distinctive "J" element which bore two very large satellites arranged in tandem. These in the equatorial-plate stage are shown by five split elements in text fig. 59, and by five anaphase sister pairs in figure 60. No other plant which the

writer has examined has yielded as many scores of beautifully unobscured cases for study. This element has been seen as a single split one only at the equatorial-plate stage, and it is so easily recognized that if present in the diploid state it could not fail of recognition. Its homologue is probably a



TEXT FIGS. 59, 60. Root-tip mitoses, *Allium Cepa*. FIG. 59. Five examples of split equatorial-plate chromosomes with tandem satellites. FIG. 60. Five examples of anaphase sister chromosomes with tandem satellites.  $\times 2000$ .

"J" element without any satellites, but difficulty was found in establishing this clearly. The origin of the material of this peculiar type has been sought without success. There have been examined seedling root tips of the forms Prizetaker, Gigantic Gibraltar, White Queen, White Portugal, Red Wethersfield, Mammoth Silver King, Ideal Yellow Globe, Yellow Globe Danvers, Southport Yellow and Southport White Globes, as well as root tips from bulbs of White Multiplier and from what passes as a large Bermuda onion in the Philadelphia market, and from several lots of bulbs of the Globe type. All these several types of commercial onion showed the two "J" elements with single small satellites. In addition, a few types of leeks and of showy-flowered onions have been examined, but these gave no data at all of use here. Consequently no opinion may be ventured as to the origin of the material described.

#### BRYOPHYLLUM CALYCINUM SALISB.

In the search for plants showing satellites on the chromosomes the writer has examined more or less closely over 70 species belonging to probably over 50 plant genera. Much of this material has proved to be without interest from the standpoint of the major problem. Of this, much is not worth

further study, some of it is being studied further by students, and for some a brief statement may be made of the observations recorded.

*Bryophyllum calycinum* appears not to have been investigated cytologically. A study of the root-tip cells shows that the chromosomes are very small, somewhat pointed, curved, and unlike in size at the equatorial-plate stage (text fig. 48). The diploid number is probably 40, possibly only 38.

#### FREESIA REFRACTA KLATT.

This plant shows in the horticultural variety "Fisleri" 22 small, somewhat irregular, rod-shaped chromosomes (text fig. 49). It has apparently not previously been reported upon.

#### RICINUS COMMUNIS L.

This plant has been studied by Němec, whose book is not available, and by Suessenguth (21) who simply confirms Němec's findings. These writers report the chromosome number as  $n = 10$ . A study of root-tip divisions has confirmed this with a count of  $2n = 20$ . The chromosomes are small, slightly tapering rods, usually curved in the equatorial plate (text fig. 54).

#### SUMMARY

A general review of the phenomena of satellites and of chromosome constrictions indicates that these features are important hereditary morphological characters of the chromosomes.

*Fritillaria imperialis* shows two pairs of metaphase chromosomes bearing distal satellites of different sizes, and a constant assortment of other chromosomes with submedian or subterminal fiber-attachment.

*Alstroemeria braziliensis*, with 8 pairs of chromosomes, shows one metaphase pair bearing small proximal satellites and five other types of chromosomes with median or subterminal fiber-attachment.

*Silphium perfoliatum* shows 7 pairs of metaphase chromosomes of which one pair may bear satellites, the remainder being divided into five classes on the basis of fiber-attachment constrictions.

*Allium Cepa* presents a rare abnormality in which a single "J"-shaped element is present bearing tandem satellites on the shorter arm.

In the diploid phase *Bryophyllum calycinum* appears to have 40 (38?) chromosomes, *Freesia refracta* has 22, and *Ricinus communis* has 20.

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<sup>1</sup> In Russian. Known only as abstracted by Sorokine (18).

# THE EFFECT OF TRANSPIRATION AND ENVIRONMENTAL FACTORS ON LEAF TEMPERATURES I. TRANSPIRATION

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It is often stated in textbooks and elsewhere that transpiration is beneficial to plants because it cools the leaves and thus prevents burning in bright sunlight. Some work on the effect of transpiration upon leaf temperatures has been done, but it has not been demonstrated that the slight cooling effect due to transpiration is sufficient to protect the leaves from injury under intense insolation. To determine more accurately the cooling effect of transpiration and to determine whether or not this might be beneficial to the plant, the work reported in this and a following paper was undertaken in the summer of 1922. Furthermore, it was thought that a correlation might be found between the rate of transpiration and the difference between the leaf temperature and that of the air, or between two leaf temperatures; so that the transpiration rate of a leaf might be estimated by comparing its temperature with that of other leaves or with the air temperature.

## HISTORICAL

The temperature of plants has been studied by many investigators. The earliest workers used mercury thermometers pressed against the leaves, or wrapped the leaves about the thermometers; and that method has been used to the present day, especially when quick approximate results have been desired. More accurate determinations, however, can be made by means of thermocouples. Probably the first to apply this method to the study of plants was Becquerel, in Paris. His first experiment, performed in 1837, was not a success, however, and his results were not published. Then Van Beek and Bergsma (25), using thermocouples in 1838, found on one occasion the spadix of *Colocasia odora* to be 22° C. warmer than the air. Dutrochet (7) in 1840 found the club-like portion of the spadix of *Arum maculatum* to be 10.4° C. warmer than the air an hour and a half after the opening of the spathe. Living stems of *Campanula media*, *Sempervivum tectorum*, and cactus, however, were as a rule about 1° C. cooler than the air except when exposed to the sun. The difference he thought to be due to the cooling effect of transpiration.

Askenasy (1) measured the temperature of species of *Sempervivum* in sunlight by means of a mercury thermometer held against the surfaces of the leaves. Many of the temperatures were between 48° and 50° C., and one reading was 52° C. which was 24° above the air temperature. Ewart (9)

found the temperatures of leaves of *Hoya fraterna* in the tropics to be about 45° C. in the sun, and sometimes the temperature went over 50° C. Neither of these men reported any burning of the plants in sunlight, although Askenasy suggested such a possibility on the basis of Sachs' work (18). Stahl (23) and others have measured the temperatures of variegated leaves and found the red and white portions of a leaf in sunlight to be at a different temperature from that of the green portions.

Ursprung (24) found the temperature of *Sempervivum*, as measured by a mercury thermometer, to range over 36.6° C. in one day between early morning and 2:00 p.m. *Betula* and *Ulmus* ranged over 13° and 14° C. respectively. On one bright day the temperature of an *Opuntia* plant was 17.3° C. above the air temperature, while a *Sempervivum* leaf was 23.6°, and a *Betula* leaf only 2.3° C. above the air temperature. Smith (22), however, using thermocouples, reported that thin- and thick-leaved plants both reached 13° to 16° C. above the air temperature in still air in Ceylon. But the temperature of the thin-leaved plants fluctuated more rapidly.

Seeley (19) in Michigan found the leaves of *Fragaria vesca* wrapped around a mercury thermometer to show 14° C. above the air temperature in June, and 7.5° warmer at noon on a day in March when the ground was covered with snow. Only on 41 dark, cloudy days out of the 304 days on which readings were taken were the leaf temperatures below those of the air at midday.

MacDougal (15) has found that an *Opuntia* stem would continue to grow until its internal temperature reached 58.3° C., and that it could be heated further to 62° C. without injury. Recently Johnson (12) has shown the southeast side of the giant cactus, *Carnegiea gigantea*, to average 3.71° C. warmer than the west side during a single day. Both of these latter two workers used thermometers.

Harvey (10, 11) found by means of thermocouples that the cambium on the south side of a tree was much warmer than that on the north side in bright sunlight in winter, and was subject to rapid fluctuations in temperature. With the passing of a cloud the temperature of a black plum branch rose 10° C. in three minutes. He found that the color of the bark affected the temperature: a white birch stem in sunlight was 1.5° C. above the air temperature, and a Dart apple stem with the same exposure, 5.1° C. warmer than the air. At one time the cambium of a black Jewel Plum was 10° C. warmer than the air.

These data show some of the temperatures normally attained by plant tissues in sunlight. A number of workers primarily interested in photosynthesis have also made observations of leaf temperatures under the conditions of their experiments. Miss Matthaei (16), using thermocouples, reported the leaves of cherry laurel to be 8°, 12°, and even 17° C. warmer than the water bath surrounding the leaf chamber, when she used the brightest illumination. Blackman and Matthaei (2) observed a consider-

able difference in the temperature of a leaf when it was placed at different angles to the sun's rays, and an abnormal rise to  $51^{\circ}$  C., with final burning, when the leaf was enclosed in a glass box. They also noticed rapid fluctuations of temperature when the leaf was shaded and exposed to sunlight. Brown and Escombe (3) calculated the leaf temperature of *Helianthus annuus* from their measurements of the energy falling upon and used by the leaf. The highest estimate they give is only  $1.54^{\circ}$  C. above the air temperature. Ehlers (8), by means of thermocouples, found the leaves of *Pinus laricio austriaca* to be  $2^{\circ}$  to  $10^{\circ}$  C. warmer than the air during the day in winter. At night they usually were  $0.1^{\circ}$  to  $0.7^{\circ}$  cooler. He also plotted the rapid fluctuations of temperature.

Some workers have measured leaf temperatures in relation to transpiration rates. The greatest temperature difference between a green and a withered leaf of *Tropaeolum majus* in sunlight observed by Darwin (5), by wrapping the leaves about a thermometer, was  $3.9^{\circ}$  C. In another experiment he (6) obtained a difference of  $1.5^{\circ}$  C. between a withered and a living leaf of *Campanula pyramidalis* by means of a resistance thermometer. Smith (22) found the temperature of a dead brown leaf in sunlight to rise to  $50^{\circ}$  C., or  $23^{\circ}$  above the air temperature. Putting two leaves together with their stomatal surfaces inward, he found them to average  $2.2^{\circ}$  C. warmer than similar leaves with their stomatal surfaces outward. Temperatures of leaves with transpiration thus checked rose to  $45^{\circ}$  C. and the leaves turned brown.

Mrs. Shreve (20), at the Desert Laboratory, used a thermocouple held against the leaf by a spring clamp, but to prevent unequal heating of the parts of the circuit she shaded the entire apparatus during the determination of the temperatures. The greatest differences between the leaf and the air temperatures which she observed (21) by means of a junction clamped between a leaf and a strip of hygrometric paper were  $1.5^{\circ}$  C. for *Encelia farinosa* in the open, and  $1.3^{\circ}$  for *Tradescantia* in the greenhouse. A possible criticism of Mrs. Shreve's method, and of that used by Miller and Saunders (17) is given below, under the heading "Discussion."

Miller and Saunders (17) measured the temperatures of leaves of a number of crop plants by means of a thermocouple similar to that used by Mrs. Shreve. They found wilted leaves, which were transpiring less rapidly than turgid ones, to be warmer than the turgid ones. Wilted corn leaves averaged  $1.85^{\circ}$  C. warmer; sorghum,  $1.55^{\circ}$  warmer; soybeans,  $2.8^{\circ}$  warmer; and wilted cowpeas,  $4.65^{\circ}$  C. warmer than the respective turgid leaves. The turgid leaves of cowpeas and of alfalfa were constantly cooler than the air even in sunlight.

This review of the literature shows that the temperature of plant tissues is usually higher than that of the air in bright sunlight, but that it is subject to wide and rapid fluctuations. From the available evidence, however, the cooling effect of transpiration seldom exceeds  $2^{\circ}$  or  $3^{\circ}$  C.

## APPARATUS AND METHODS

The leaf temperatures in these experiments were determined by means of thermocouples inserted into the mesophyll, or sometimes into the midrib, of the leaf under experiment. All the electrical apparatus except the junctions was made by the Leeds and Northrup Company of Philadelphia. The junctions were made by the writer by fusing, with an electric current, the ends of no. 36 copper and constantan wires, which had been obtained from Leeds and Northrup. Three-foot lengths of these wires were connected in parallel to one wire of the 110-volt electric-lighting system. To the other wire of the lighting circuit was attached a resistance coil of 25.5 ohms. Holding the free ends of the copper and constantan wires together and touching these to the pole of the resistance coil for only an instant, a spark, hot enough to fuse the wires, was formed when the contact was broken again. The contact and break were made with a single quick motion of the hand. When a satisfactory junction was obtained it was filed down with emery paper until it was no larger than the diameter of the two wires together. Next the wires were insulated with "Radiolac." The copper wire was cut in the middle, and the ends were wound around the ends of no. 20 copper wires and soldered in place. The no. 20 wires went to the multiple switch and potentiometer. The no. 36 wires were further protected by running them through cotton sleeving. This kept the pairs of wires together and greatly facilitated handling.

The usual apparatus of potentiometer, galvanometer, storage battery, and standard cell was employed. The potentiometer was a Leeds and Northrup type K high-precision potentiometer, and the galvanometer, from the same firm, was type R, no. 2500 e, with a sensitivity of 0.5 micro-volt. A multiple switch was placed between the thermocouples and the potentiometer so that readings could be taken from several junctions in rapid succession. All the apparatus was placed on a bench in the greenhouse and protected from the sun by a canopy of black oilcloth with black curtains on all sides. The wires from the thermocouples to the multiple switch were long enough to reach to the south end of the greenhouse, where the plants were placed in direct sunlight, and in the summer they were extended so that the plants could be placed on a table or on the ground out of doors. In all the experiments the cold junctions were kept in a Dewar flask containing ice and distilled water. The ice would keep in this flask for three or four days even when it was exposed to the sun during the day, but to insure the maintenance of a constant temperature at 0° C., the flask was kept full of ice.

To calibrate the apparatus a large standardized mercury thermometer, which was graduated to tenths of a degree centigrade, was used. The cold junctions were placed in the Dewar flask containing ice and distilled water. The other junctions of all the couples were bound to the bulb of the mercury thermometer and placed in another Dewar flask containing water. This



flask was fitted with a siphon and a glass stirring rod. The water at the beginning of the calibration was about  $53^{\circ}\text{C}$ . As soon as the thermometer showed a uniform temperature, the potentiometer was balanced, using the first thermocouple. Then the thermometer was read, estimating to hundredths of a degree, and both readings were recorded. Readings of all the thermocouples with corresponding thermometer readings were obtained in the same way. Some of the water was next siphoned off and enough cold water was added to reduce the temperature of the whole one or more degrees. The water was stirred and, as soon as the thermometer had again become stationary, readings were taken at that temperature. In this way readings were taken from  $50^{\circ}$  to  $10^{\circ}\text{C}$ . Later the instrument was calibrated down to  $-5^{\circ}\text{C}$ . The readings were plotted on coördinate paper, microvolts on the abscissae and degrees on the ordinates, and a curve was drawn through the majority of the points. The curve was nearly a straight line. All the readings of all the couples fell within one tenth of a degree of the line. In using the apparatus and translating microvolts to degrees, the microvolts were recorded as read from the potentiometer, and later these readings were changed to temperature values simply by locating the potentiometer readings on the curve.

The junctions were inserted into the mesophyll of the leaf whenever possible. A small hole through the lower epidermis was made with a fine needle, and the junction was pushed far enough into this so that the fused portion of the wires was buried between the upper and the lower epidermis. Each junction was held in place with a small wire clamp slipped over the edge of the leaf and over the thermocouple wires a short distance from the junction. In most cases the leaves were held up by strings, fastened to the clamps which held the thermocouples in place, and adjusted so that the exposure of the different leaves to the sun was nearly equal.

TABLE I. *The Effect of Sunlight and Shade upon Thermocouples in the Air*

No. 1 One Junction in Sun, the other Shaded	No. 2 Shaded	No. 3 In Sun	Differences, 3-2	
	24.77°	26.33°	+ 1.56°	
	25.43	25.06	- 0.37	
	24.3	25.22	+ 0.92	
	25.45	25.66	+ 0.21	
	25.38	26.51	+ 1.13	
+ 0.95°	25.25	23.9	- 1.35	
+ 1.17	23.79	23.58	- 0.21	
+ 1.13	26.47	25.39	- 1.08	
	24.27	24.6	+ 0.33	
+ 0.35	24.27	24.09	- 0.18	
	23.9	24.67	+ 0.77	
+ 0.90°	24.84°	25.00°	+ 0.16°	Means
± 0.114			± 0.1759	Probable error of means

Since the junctions in the mesophyll were sometimes covered by different amounts of leaf tissue, and sometimes covered by little more than the upper epidermis, an experiment was performed to determine the heating effect of the direct rays of the sun on the thermocouples themselves. Smith (22) stated that the sun has no effect on such junctions.

Table 1 shows that the temperature of junctions 2 and 3 fluctuated a good deal, but the mean of the differences between individual readings was only  $0.16^{\circ}\text{C}$ . with a probable error of  $\pm 0.1759$ . The differences, therefore, were not significant. Comparing successive readings, however, is not altogether accurate, because the temperature of one junction may change while the reading of the second is being made. To avoid this error, thermocouple no. 1 was placed with one junction exposed to the sun and the other shaded. The table shows that the junction in the sun was always warmer, and the difference is significant. These differences in temperature between a junction exposed to the sun and one shaded by a board are small, however, compared with the differences between leaf and air temperatures, and probably the effect of the rays of the sun which pass through the cells of the leaf overlying the junctions is negligible if the junction is in good contact with the leaf tissue.

Since Shreve (20) used a junction clamped upon the surface of the leaf, it was thought that possibly as good results could be obtained in that way as from the junction in the mesophyll, and that thus much time would be saved in placing the junctions at the beginning of an experiment. For this purpose two junctions were prepared which had broad, flat contact points, and experiments were performed to determine how the readings from these junctions compared with those from other junctions inserted into the mesophyll. In general, the differences between the readings from a junction clamped against the under surface of a leaf and those from a junction inserted into the mesophyll were less than the differences between readings from opposite sides of the same leaf. But, as it was difficult to be sure that the junction on the surface always made a good contact with the leaf, the junctions were inserted into the mesophyll whenever possible in all the experiments reported in these papers.

Air temperatures were always determined by a junction, shaded from the sun, but hung free in the air near the leaves under investigation. Usually it was placed in the shadow of one of the leaves containing other junctions. In all but the first rather preliminary experiments, the relative humidity of the air in the immediate vicinity of the plants was determined by means of Lambrecht's polymeters. These polymeters were corrected every few weeks with a sling psychrometer.

The transpiration of the plants was determined in some cases by loss of weight from potted plants, the pots and soil being coated with paraffin. In order to obtain data from a single leaf, however, Ganong potometers were used. Instead of the usual rubber stopper, the leaves were sealed into the

potometers with plasteline, and grafting wax was used to make a tight seal between the petiole of the leaf and the plasteline. All the transpiration rates reported in this paper have been expressed in grams, or cubic centimeters, per square decimeter of leaf area per hour. The rate per unit of surface, if both surfaces of the leaf were considered, would be just half as great as the values given.

#### THE EFFECT OF TRANSPIRATION ON LEAF TEMPERATURES

As stated at the beginning of this paper, an attempt has been made to observe the differences in temperature between various leaves which were transpiring at different rates, with the hope in mind that a correlation might be found between the temperatures and the transpiration rates. The first material investigated was *Fuchsia speciosa*, growing in small pots. To make a difference in transpiration rates, one plant was watered quite heavily and another allowed to become rather dry. No attempt was made to determine the water content of the soil, because the main object of the experiment was not to have the plants under specified conditions of soil moisture, but simply to measure the transpiration rates and the temperatures over a certain period of time and see if a relationship could be found. The pots were coated with paraffin, weighed, and the thermocouples were inserted into the leaves. At the end of the experiment the thermocouples were removed and the pots were again weighed.

In the first experiments performed in the summer of 1922, one leaf was chosen on each plant and only one junction was used in each. Both leaves had a similar exposure to the sun. Consecutive readings were taken on the two leaves over a period of an hour, late in the afternoon. The sun was low and the differences in temperature were small, but each pair of readings showed the leaf of plant *B*, in dry soil, to be slightly warmer than the leaf of plant *A*, in wet soil. The averages of the temperature readings, the means of the differences between individual temperature readings and the probable errors of these means, and the transpiration rates are given in table 2. The temperature data in this and the following tables were treated in this way because the temperature of the leaves always varied so much during the course of an experiment that, if the mean temperature of each leaf had been obtained, the differences between the means, and the probable errors of these differences, the differences would have appeared insignificant. Yet the differences between individual readings taken at the same time were often quite constant. In some cases the mean of the differences between individual temperature readings is not the same as the difference between the averages of the same temperature readings, so that the values given for the differences are not always the exact differences between the average temperatures shown. The differences in temperature are represented as  $B - A$ , because the leaf of plant *B*, in dry soil, was expected to be warmer than the leaf of plant *A*, in wet soil; but the differences in transpiration are

given as  $A - B$ , because plant  $A$  was expected to transpire more rapidly. On August 26, 1922, plant  $B$  showed a mean temperature difference of  $0.3^{\circ}\text{C}$ . higher than plant  $A$ , and this is a significant difference as shown by the small probable error. The transpiration rate of plant  $B$  was 0.394 gram per square decimeter per hour less than that of plant  $A$ . That is about as would be expected; a loss of 0.394 gram of water per square decimeter per hour from plant  $A$  in excess of that lost from plant  $B$  might have had a cooling effect of  $0.3^{\circ}\text{C}$ .

TABLE 2. *Leaf Temperatures and Transpiration Rates of Fuchsia Plants in Pots*

	Plant A, in Wet Soil	Plant B, in Dry Soil	Differences	Probable Errors
August 26, 1922				
Leaf temperature.....	23.57°	23.87°	$B - A, + 0.3^{\circ}$	$\pm 0.0437$
Leaf temp. minus air temp.....	+ 1.05°	+ 1.35°		
Probable error.....	$\pm 0.1738$	$\pm 0.1685$		
Transpiration g. sq. dec. hr.....	0.784	0.354	$A - B, + 0.394$	
Air temperature.....	22.52°			
Relative humidity.....	46%			
August 28, 1922				
Leaf temperature.....	32.37°	30.86°	$B - A, - 1.51^{\circ}$	$\pm 0.3816$
Leaf temp. minus air temp.....	+ 6.95°	+ 5.26°		
Probable error.....	$\pm 0.2277$	$\pm 0.4817$		
Transpiration g. sq. dec. hr.....	0.722	0.196	$A - B, + 0.526$	
Air temperature.....	25.27°			
Relative humidity.....	23%			

Two days later the experiment was repeated (table 2), but this time it was performed earlier in the afternoon with the plants exposed to bright sunlight. The averages are shown as before. The leaves were considerably warmer because of the bright sunlight. The transpiration rate of plant  $A$ , in wet soil, was about the same as before, while that of plant  $B$ , in dry soil, was much less. But in this case plant  $A$ , which was transpiring more rapidly, also averaged  $1.51^{\circ}\text{C}$ . warmer, with a probable error of only  $\pm 0.3816^{\circ}$ . The results were just the opposite of those expected. The relative humidity in this case was much less, but apparently that fact did not increase the transpiration rates. Although the attempt was made to give both leaves the same exposure, the one on plant  $A$  may have been at a more favorable angle to receive the sun's rays, and this may have been the reason for its higher temperature.

The following summer three experiments were performed with potted Fuchsia plants, but the transpiration data of two of these were unreliable. The average temperatures and the transpiration rates of the third are presented in table 3. Two plants in dry soil in paraffined pots were used.

On both plants two leaves with similar exposure were selected, and a thermocouple was placed in each. The plants were then weighed with the junctions in place. After taking a number of readings, one leaf of each plant which contained one of the junctions was vaselined on the under surface to check the transpiration of that leaf. More readings were made. At the end of three hours both plants were weighed, and plant *A* was watered and weighed again. Temperature readings were taken during a second period of three hours, and the plants then reweighed. The water was added to plant *A* to increase the transpiration rate of that plant during the experiment, so that, if increasing the transpiration changed the temperature relations between that plant and the other, or between that plant and the air, the fact could be observed. A half hour after the water was added, plant *A* became noticeably more turgid than it had been before watering. The water was added at noon so that the sun would be at the same angles during both periods of the experiment. The plants were turned from time to time

TABLE 3. *Leaf Temperatures and Transpiration Rates of Fuchsia Plants in Pots, July 12, 1923 (Weather: Bright and Clear, Moderate Breeze)*

	Plant A			Plant B			
	Leaf 1	Leaf 2	Diff., 2-1	Leaf 3	Leaf 4	Diff., 3-4	
1st period:							
Leaf temp.....	30.86°	28.89°	-1.54°	29.62°	29.9°	-0.2°	
Prob. error.....			±0.1462			±0.1296	
2d period:		Vaselined		Vaselined			
Leaf temp.....	32.69°	31.2°	-1.49°	32.18°	31.92°	+0.26°	
Prob. error.....			±0.3704			±0.2737	
3rd period (Watered plant A):							
Leaf temp.....	31.79°	33.02°	+1.23°	32.18°	30.72°	+1.46°	
Prob. error.....			±0.1795			±0.2255	
	Plant A	Plant B	Dried Leaf F	Mean Diff., F-A	Mean Diff., F-B	Air Temp.	R. H.
1st and 2d periods:							
Leaf temp.....	31.22°	30.4°	33.48°	+3.33°	+3.90°	25.78°	42%
Leaf temp. minus air temp.....	+5.25°	+4.56°					
Prob. error.....	±0.3578	±0.4489		±0.3624	±0.5039		
Transpiration g. sq. dec. hr.....	0.0458	0.0939					
Temp. diff., B - A ...	-0.47°	±0.1900					
Transp. diff., A - B ..	-0.0481						
3rd period (Watered plant A):							
Leaf temp.....	31.79°	30.72°	35.73°	+4.21°	+5.34°	28.53°	36%
Temp. diff., leaf - air..	+3.28°	+2.5°					
Prob. error.....	±0.277	±0.2025		±0.5471	±0.4411		
Transpiration g. sq. dec. hr.....	0.1837	0.0939					
Temp. diff., B - A ...	-0.92°	±0.2255					
Transp. diff., A - B ..	+0.0898						

so that the sun always shone directly on the leaves containing the junctions. A pressed dried Fuchsia leaf which had retained nearly its normal green color was clamped in such a position that it received an exposure to the sunlight similar to that of the experimental leaves of the plants. The temperature of this leaf was determined by means of a junction clamped to the under surface.

In the first part of table 3 the temperatures of two leaves of each plant are compared. Between the first and second periods leaves 2 and 3 were vaselined. At first both these leaves were cooler than the other experimental leaves of the respective plants, but the means of the temperature differences are not significant in the case of plant *B*. The probable errors in this table, as in those preceding, are the probable errors of the means of the differences between individual temperature readings. During the third period the vaselined leaves were warmer than the other experimental leaves, and the differences, although less than  $1.5^{\circ}$  C., appear significant. From the latter part of the table it will be seen that the dried leaf was 3 to 5 degrees warmer than the green leaves, and the differences are all significant.

Comparing the temperatures of the unvaselined leaves of the two plants and the transpiration rates, it will be seen that, during the first two periods of the experiment, plant *A* was transpiring only about half as fast as plant *B*, and it was slightly warmer, although the difference in temperature was hardly significant. After watering, plant *A* transpired more rapidly, but it maintained even a greater temperature difference over plant *B*, so that no correlation could be drawn between the differences in temperature and the differences in transpiration rates.

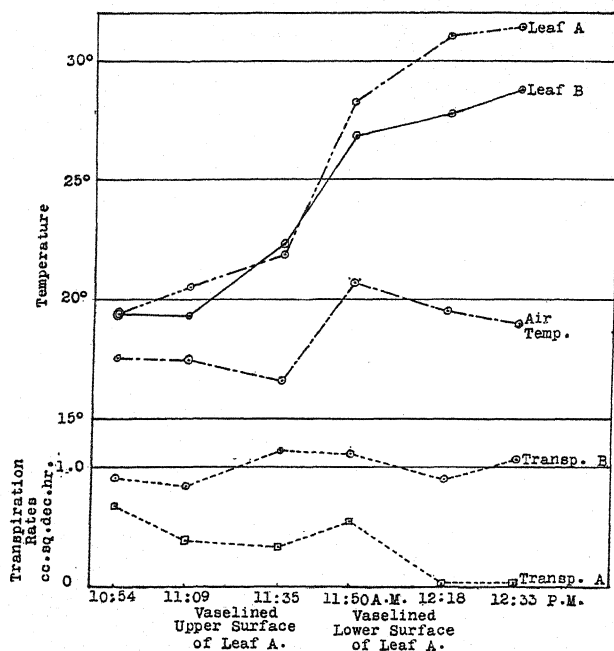
From these results with Fuchsia plants it seems that, when transpiration is greatly reduced, as when the leaf is vaselined, the leaf does become a little warmer than an unvaselined one, and also that a dried leaf is considerably warmer than a normal one. But small changes in transpiration rate have little effect upon the leaf temperature, and the effect is in no way constant.

During the fall of 1922 experiments were carried out in the greenhouse, using the "Nova Scotia Marl" variety of wax bean, *Phaseolus vulgaris* L., grown in pots. The data from these experiments are not shown, but they were similar to those from Fuchsia plants. No definite relationship was evident between the differences in transpiration rates and the differences in leaf temperatures. Sometimes the leaves of a plant which was transpiring more rapidly than another were slightly cooler than those of the second plant, as would be expected, but sometimes they were warmer. The mean differences were always small, and sometimes insignificant.

Experiments similar to those performed with Fuchsia were carried out with cabbage plants, *Brassica oleracea* L. The variety was "Copenhagen Market." To prevent transpiration by vaselining, both surfaces of a leaf had to be coated, because cabbage has stomata in both the upper and the

lower epidermis. It was feared that vaselining the upper surface might seriously change the amount of heat absorbed by the leaf. To test this possibility, two similar leaves from the same plant were placed in potometers in bright sunlight and their temperature and transpiration rates were observed for half an hour. Then the upper surface of one leaf was vaselined, and readings were taken during the next half hour. Finally the lower surface of the same leaf was vaselined, and observations were made as before. The potometers were read every fifteen minutes, and the transpiration rates were calculated in cubic centimeters per square decimeter of leaf area per hour. Four or six temperature determinations of each leaf and also of the air were made between each 2 successive readings of the potometers.

In text figure 1 the temperatures and transpiration rates are plotted on



TEXT FIG. 1. The effect of vaselining the upper surface and then the lower surface of a cabbage leaf as indicated by the changes of temperature and of transpiration rate. Leaf A vaselined at the times indicated, leaf B not vaselined. May 22, 1923. Relative humidity, 34 percent to 37 percent.

the ordinates and the time in intervals of fifteen minutes on the abscissae. The few minutes necessary to reset the potometers when the bubble of air had traveled nearly the length of the capillary is not taken into consideration, so that the times indicated at the bottom of the graph are not always 15 minutes apart but are the exact times at which a reading of one of the potometers was made, and in each case the transpiration rate indicated

above that time is the rate calculated for the 15 minutes preceding the given time. The temperatures are the averages of the readings made during the same 15 minutes. The time which elapsed while the leaf was being vaselined is also not represented accurately, but the graph is not meant to follow precisely the changes of leaf temperature with time. It simply indicates the changes in leaf temperatures and transpiration rates under different conditions.

Before vaselining either surface of leaf *A*, it averaged respectively  $0.03^{\circ}$  and  $1.24^{\circ}$  C. warmer than leaf *B* during the two intervals of fifteen minutes. After vaselining the upper surface only, it averaged  $0.36^{\circ}$  cooler in the first period and  $1.45^{\circ}$  warmer in the second period, and the transpiration rate did not change materially. But when the lower surface was also vaselined, leaf *A* became  $3.29^{\circ}$  and  $2.64^{\circ}$  C. warmer than leaf *B*. From these data it would appear that vaselining the upper surface of the cabbage leaf had little effect upon the amount of heat absorbed from the sun, and little effect upon the transpiration rate. When the lower surface was also vaselined the transpiration was cut down greatly, and the temperature difference between the vaselined and the normal leaf rose about two degrees.

The data from potted cabbage plants are given in table 4. The averages are shown of many temperature readings taken from each plant on different days under the conditions stated. The temperatures of vaselined leaves and of a dried leaf are given, as well as the transpiration rates of the plants and the differences between leaf temperatures and between transpiration rates. The temperature differences are the means of the differences between individual temperature readings of the various leaves and of the air. On June 22, 1923, plant *A*, in moist soil, was transpiring faster than plant *B*, in dry soil, by 0.7492 g. per sq. dec. per hr., and was  $1.63^{\circ}$  C. cooler. In the first part of the experiment performed on June 25, the soil conditions were the same as in the previous experiment. Plant *A* was transpiring 1.1183 g. per sq. dec. per hr. faster than plant *B*, but this time it averaged only  $1.08^{\circ}$  C. cooler. At noon plant *B* was watered, and its transpiration rate was increased. The difference in transpiration rates between the two was less than in the morning, but the difference in temperature was half a degree greater. This was the opposite of what would be expected. June 26 was partly cloudy, and the temperature differences were so small that the means of the differences between the leaf and the air temperatures do not appear significant for either plant *A* or plant *B*. Yet plant *B*, in dry soil, was really transpiring more rapidly than plant *A*, and was cooler by a small but significant difference. On July 2, plant *A* was transpiring 0.619 g. per sq. dec. per hr. faster than plant *B* and was cooler by only  $0.14^{\circ}$  C., but this difference was not significant. By noon plant *B* became wilted and water was added. After noon the transpiration rate of this plant was only about half as great, but it became turgid almost immediately after watering. In the afternoon the difference in transpiration rate was greater than it had



been in the morning and the difference in temperature was also greater, but there was nothing proportional about these larger differences. Neither did they correspond to the differences in transpiration rates and temperatures obtained on any other day. On July 7 the soil of both plants was quite dry to start with, but that of plant *B* was more dry and this plant wilted by noon. During the morning there was a greater temperature difference than on any preceding day shown in this table, but the difference in transpiration was rather small. After watering both plants at noon, the transpiration rates of both increased and the differences between the rates became greater, but the difference in temperature was  $1.10^{\circ}$  C. less. July 11 began bright but later became partly cloudy, and there was such a great difference in leaf temperatures in the sun and when shaded by clouds that the temperatures under the two conditions of light intensity were averaged separately. Plant *B*, in dry soil, was transpiring faster during the morning than plant *A*, and it was considerably cooler both in the sunlight and under the cloud. It wilted by noon but revived a half hour after watering. During the afternoon it transpired much more slowly, more slowly than plant *A*, yet plant *A* was constantly warmer, both in the sun and when shaded by a cloud. Only one set of readings, however, was made while it was cloudy.

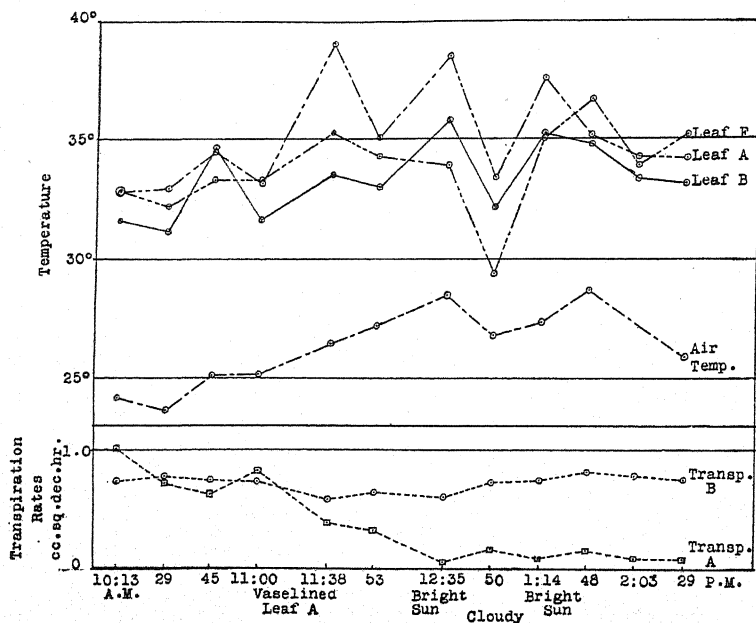
These data from cabbage plants bring one to the same conclusion as the data from *Fuchsia*: namely, that there is no definite correlation between the transpiration rate and the temperature of the leaves of a plant. For a plant which is transpiring more rapidly may not always have a lower temperature than one transpiring more slowly, and even when it is cooler the difference is small compared with differences caused by other factors.

Table 4 also gives the temperatures of leaves of these same plants which were vaselined either during the experiment or some time previously. The leaves were so selected and the plants so arranged that all four of the leaves whose temperatures were taken had as nearly as possible the same exposure to the sun. In the first case, that of June 22, each of the two leaves which were later vaselined was cooler than the other experimental leaf of the same plant. Although they were placed as nearly equally as possible, the angle at which the sun's rays struck these leaves may not have been the same. This difference may have caused the difference in temperature at first. After vaselining, one leaf was  $0.87^{\circ}$  C. and the other  $1.73^{\circ}$  warmer than the corresponding unvaselined leaves. Here the prevention of transpiration with vaseline allowed the leaves to become warmer in sunlight. In the next case, that of June 25, the leaves were vaselined throughout the experiment. One was warmer and the other cooler than the unvaselined leaf on the same plant, but the mean differences were not significant. In most of the other cases, but not in all, the vaselined leaves were warmer. In cases in which they were not warmer the correct explanation was uncertain. The difference might have been due to a difference in light intensity caused by an unavoidable inequality in the angle at which the leaves were set.

TABLE 4. Leaf Temperatures and Transpiration Rates of Potted Cabbage Plants (Summer, 1923)

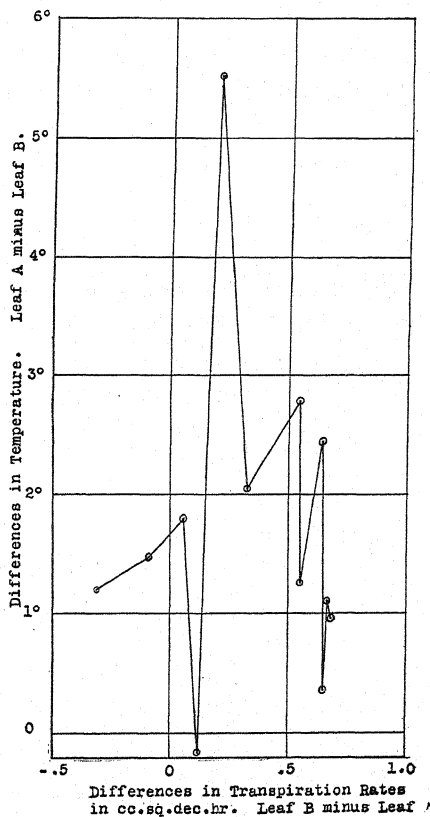
The vaselining, however, did not produce a great increase in temperature, and it seems unlikely that transpiration exerted a very great cooling effect.

The columns at the right of the table give the means of temperature readings taken from a pressed, dried cabbage leaf which had nearly the normal green color, and the means of the differences between the readings from this leaf and from the normal green leaves. Sometimes the dried leaf was 2 to 3 degrees warmer than the normal leaf, but frequently the difference was less than one degree, and insignificant. Since the mean temperature difference between a perfectly dry leaf and a normal green one, for any one experiment, was only about  $3^{\circ}\text{C}$ . under the most extreme conditions encountered during the summer of 1923; and, furthermore, since the highest temperature recorded for the dry leaf was  $45^{\circ}\text{C}$ . and the highest for the normal green leaf on the same day was  $44.2^{\circ}$ , it would seem that the cooling effect of transpiration was too small to be of benefit to the plant. Certainly it did not protect the leaves from injury, because even the dried leaf did not reach a temperature high enough to injure a normal leaf. It is true that above  $40^{\circ}\text{C}$ . the rate of photosynthesis would fall off rapidly, as shown by Matthaei (16), but the temperature of the green leaf which was free to transpire also rose above  $40^{\circ}\text{C}$ .



TEXT FIG. 2. Temperature and transpiration rates of cabbage leaves. Leaf A vaselined at the time indicated, leaf B not vaselined, leaf F dried. July 7, 1923. Relative humidity, 32 percent to 52 percent.

In each of the experiments so far reported in this paper the temperature of one or two leaves in direct sunlight was taken as representative of the temperature of the plant, and the transpiration rates were calculated from the loss of water from the whole plant. But obviously many of the lower leaves on the north side of the plant were shaded, and others which were in the sun were at such an angle that they probably did not absorb nearly as much heat as the leaves in which the junctions were placed. These leaves would all be cooler than the leaves under experiment, and may have been transpiring at different rates. To obtain more accurate transpiration data on the leaf in which the thermocouples were placed, and also to measure the transpiration rate over shorter periods of time, Ganong potometers were used. It was known that Lloyd (13) had shown large discrepancies to occur between transpiration data obtained from potometers and those obtained from loss of weight, because the potometer measures the amount of water absorbed and not the actual amount of transpiration, but for the



TEXT FIG. 3. Curve showing the lack of correlation between the differences in temperature and the differences in transpiration rate between two cabbage leaves. July 7, 1923.

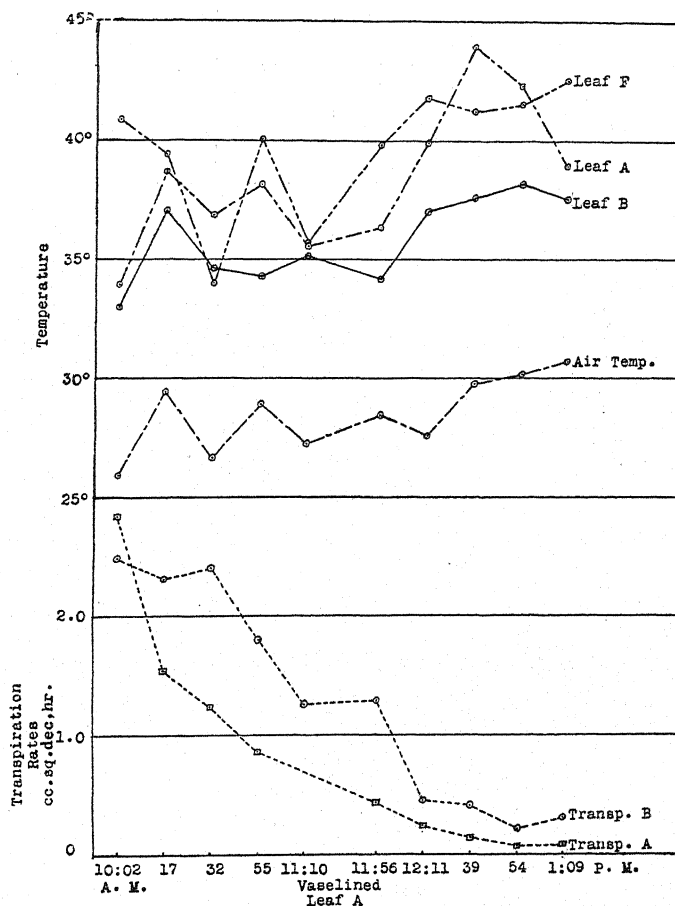
purpose of these experiments the potometer seemed the best method available. Similar leaves from the same plant were selected, and their petioles were cut under water. The living leaves used in the following experiments were designated "A" and "B," and "A" was usually vaselined during the experiment. A pressed dried leaf of the same species which had retained practically its normal green color was also used. This was labeled "F." A large number of such experiments with leaves in potometers were performed, but the results of only a few are shown graphically since they were all similar.

Text figure 2 is typical of the variations in leaf temperatures and transpiration rates during an experiment. The data were taken on a clear day in July, 1923, when there was only a little breeze. The temperature readings were averaged for 15-minute periods and located on the ordinates. The transpiration rates calculated for each 15-minute period are also indicated on the ordinates and the time intervals on the abscissae. Cabbage leaves were used. The experiment was run for an hour before leaf A was vaselined. After vaselining, the temperature of this leaf went rather high. At this time there was the greatest difference in temperature ever observed between a vaselined and an unvaselined cabbage leaf. It averaged  $5.53^{\circ}\text{C}$ . for the 15-minute period. The reason for the drop in leaf temperature during the next 15-minute period is not known. It may have been due to stronger air currents. But the drop in all of the curves shown at 12:50 p.m. was due to a cloud shading the leaves during part of the period. From this time on, the differences between the temperature of the vaselined leaf and that of the unvaselined leaf fluctuated a good deal without any corresponding changes in transpiration rate. It will be noted that at no time did the dried leaf average more than  $2^{\circ}\text{C}$ . warmer than the unvaselined leaf. Why it should be actually cooler on four occasions is not known.

Plotting the differences in temperature between the two leaves against their corresponding differences in transpiration rate gives a very widely fluctuating curve from which no correlations can be drawn. This curve is shown in text figure 3. Curves similar to those shown in text figures 2 and 3 could be shown from other experiments with cabbage leaves, but these two are representative. Thus, from experiments with individual cabbage leaves as well as from those with potted plants, one is forced to the same conclusion: namely, that there is no correlation between transpiration rates and leaf temperatures alone. Other factors are more important than transpiration in determining the temperature of leaves.

Text figures 4 and 5 show similar results obtained in an experiment with lilac leaves, *Syringa vulgaris* L., in potometers. A pair of opposite leaves was selected and treated as were the cabbage leaves in the preceding experiments. The results were averaged for 15-minute intervals and plotted. The day was clear and bright with a light breeze, and wide fluctuations in temperature occurred as in the preceding experiments. Leaf A was con-

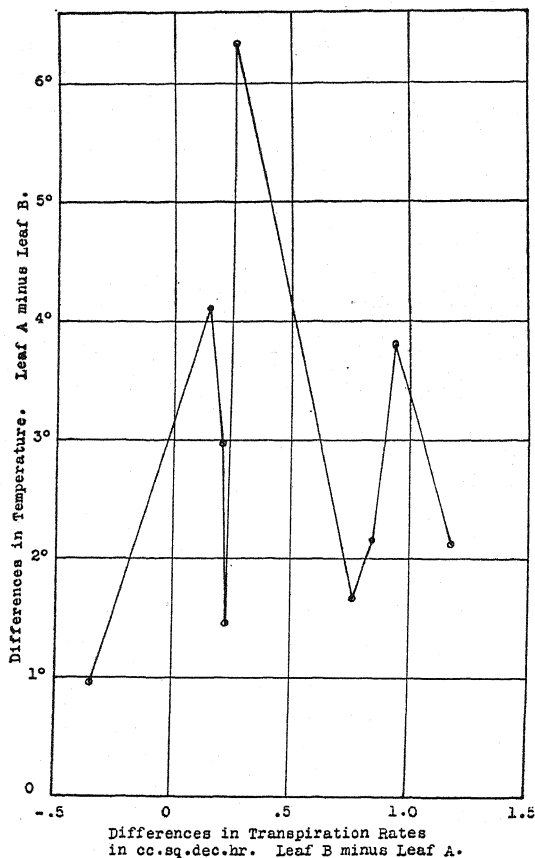
stantly warmer than leaf *B* even before the under surface of the former was vaselined, but the fluctuations were so great that no particular heating effect could be attributed to the vaselining. The pressed dried leaf was in nearly



TEXT FIG. 4. Temperature and transpiration rates of lilac leaves. Leaf *A* vaselined at the time indicated, leaf *B* not vaselined, leaf *F* dried. July 13, 1923. Relative humidity, 30 percent to 40 percent.

all cases 3 to 5 degrees warmer than the unvaselined one. No satisfactory explanation could be found for the two or three instances when this was not true, when the temperature of the pressed dried leaf fell approximately to that of leaf *B*. The transpiration rates of leaves *A* and *B* fell off rapidly from the beginning of the experiment, possibly in consequence of the closure of the stomata or of a plugging of the cut vessels, so that no great difference in transpiration was evident as a result of vaselining. In fact, the differences

became less toward the end. Plotting these differences against their respective temperature differences in text figure 5 again shows no correlation between the two.



TEXT FIG. 5. Curve showing the lack of correlation between the differences in temperature and the differences in transpiration rate between two lilac leaves. July 13, 1923.

### DISCUSSION

In these experiments, potted plants of Fuchsia and cabbage have been used, and cut leaves of cabbage and lilac, and in no case was there a definite correlation between the transpiration rate of a leaf and the difference between its temperature and that of the air, nor between the differences in transpiration rates of two leaves and the differences in their temperatures.

Brown and Wilson (4) have devised an equation for determining the thermal emissivity of a leaf from the difference in temperature between two leaves, the difference in their transpiration rates, and the heat of vaporization

of water; or from the difference between the leaf and air temperatures and the transpiration rate of the leaf, and the heat of vaporization of water. They state that the equation can be changed and used to determine the transpiration rate from the temperature difference and the emissivity; or to find the temperature difference from the transpiration rate and the emissivity. The leaves with which they determined the thermal emissivity were enclosed in a dark box and were cooler than the air. The present writer had hoped to derive a similar equation which might be used under field conditions, but no constant correlation could be found between the various factors. Besides, the temperature of the leaf is so influenced by the intensity of the light that one can tell very little about the transpiration rate from the temperature and the emissivity as calculated for leaves in the dark. It may be significant that the temperatures of leaves in sunlight calculated by Brown and Escombe (3), using the thermal emissivity which had been derived from the formula of Brown and Wilson (4), are all much lower than the temperatures found by most other investigators, including the present writer. In the experiments reported in this paper, no attempt was made to determine the amount of energy absorbed by the leaf, and so data are not at hand with which to test the formula of Brown and Escombe against leaf temperatures determined by thermocouples.

Askenasy (1) and Ursprung (24) reported leaf temperatures of *Sempervivum* in sunlight over  $20^{\circ}$  C. above that of the air. Ursprung (24) and MacDougal (15) found *Opuntia*  $17^{\circ}$  C. warmer than the air. Such temperatures are higher than any experienced by the present writer with plants in the open, but *Sempervivum* is a thick-leaved plant and *Opuntia* has a fleshy stem, and they may be of such a nature that they do not lose heat rapidly by transpiration, convection, or radiation. Smith (22) reported both thick and thin leaves in the sun at  $12^{\circ}$  and  $16^{\circ}$  C. above the air temperature, and Seeley (19) found the leaves of *Fragaria vesca*  $14^{\circ}$  C. above the air temperature on some days. These temperatures are also higher than those commonly found in the plants studied by the present writer, but the discrepancies may be due to different climatic conditions, and partly, perhaps, to the nature of the leaves. In the experiments reported in this paper, the leaf temperatures were commonly  $3^{\circ}$  to  $5^{\circ}$  C. warmer than the air in only moderately bright sunlight. During the summer, differences of  $7^{\circ}$  to  $9^{\circ}$  or even  $10^{\circ}$  C. were not infrequent. In one case the difference was  $13.1^{\circ}$ . In a few cases in the greenhouse, the leaf temperatures were  $14^{\circ}$  to  $16^{\circ}$  warmer than the air. Ursprung (24) found leaves of *Betula* only  $2^{\circ}$  to  $4^{\circ}$  C. warmer than the air, which is quite comparable to some of the differences found by the present writer. Only rarely were leaves in a normal condition cooler than the air during the day. This is in accord with the determinations of Seeley (19) for strawberry leaves, and of Ehlers (8) for pine leaves.

It seems remarkable that all the data coming from the Desert Labo-



ratory, and the recent work of Miller and Saunders (17) in Kansas, show such small differences between the leaf temperature and that of the air, and even leaf temperatures cooler than the air in sunlight. The maximum difference Mrs. Shreve (21) obtained was  $1.5^{\circ}\text{C}.$ ; Loftfield (14) states that an unwilted alfalfa leaf was never more than  $0.2^{\circ}\text{C}.$  above the air temperature, while a wilted one was at one time  $1.9^{\circ}\text{C}.$  warmer as determined by wrapping the leaf about a thermometer; and Miller and Saunders (17) seldom obtained temperatures of turgid leaves more than  $1^{\circ}\text{C}.$  above the air temperature. Some of the transpiration rates reported by these last authors are higher than those observed by the present writer, and it may be that under the climatic conditions of Kansas transpiration cools the leaves somewhat more than it does in New York. Yet the differences in temperature found by these authors between turgid and wilted leaves are comparable to the differences found by the writer between normal and vaselined leaves, although their differences between leaf and air temperatures are smaller. It does not seem that transpiration alone could account for these smaller differences, for, if transpiration were cooling the turgid leaves so markedly, one would expect the wilted leaves to be warmer. From this work of Miller and Saunders it would seem that alfalfa and cowpeas differ from other plants in their ability to lose heat, either by transpiration or by other means. Unfortunately, the present writer was unable to carry out any experiments on alfalfa during the last two summers.

One reason for the smaller temperature differences found by these authors may lie in their method of applying the thermocouples. Miller and Saunders (17) used a junction held in place by cork-tipped tongs. It may have been that the junction was influenced, not only by the temperature of the leaf, but also by the surrounding cork which was probably cooler than the leaf. Furthermore, the cork tip covered an area of the leaf  $3 \times 10\text{ mm.}$  and shaded this area for a short time. This shading may have lowered the temperature of the leaf, for, as will be shown in a succeeding paper, the temperature of a leaf may fall several degrees in a few seconds when it is shaded. Mrs. Shreve (20) also shaded the leaves during the actual determination of the temperature, and, although she states that this took but a fraction of a second, the shading may have had some effect upon the temperature. Loftfield (14) used only a thermometer, and one would not expect that to be as accurate as thermocouples.

From the experiments reported in this paper, it is evident that leaves with transpiration checked, by vaselining or by having the plants in dry soil, are often  $2^{\circ}$  or  $3^{\circ}\text{C}.$  warmer than leaves free to transpire, and dried leaves may be  $3^{\circ}$  to  $5^{\circ}\text{C}.$  warmer than normal leaves. Frequently, however, the differences are less than this, and they are never constant. Sometimes the leaves with reduced transpiration are cooler than the other leaves. The temperature of the leaves seems to depend far more upon the intensity of the light than upon the transpiration rate. Some striking effects of varying the

intensity of light upon leaf temperature will be shown in a subsequent paper. Whether the slight cooling effect of transpiration shown in this paper would ever protect the plant from injury from too strong insolation would depend somewhat upon the thermal death-point of the plant. Experiments to determine this point will also be reported later.

The writer wishes to express his gratitude to Professor O. F. Curtis of the Laboratory of Plant Physiology, Cornell University, who suggested the problem, for his kindly interest and encouraging direction. Acknowledgments should also be made to Professor D. B. Carrick of the Department of Pomology for suggestions and help in installing the apparatus, and to Dr. C. C. Bidwell of the Department of Physics for suggesting the method used in fusing the thermocouples.

#### SUMMARY

1. The temperature of leaves of *Fuchsia speciosa*, *Phaseolus vulgaris*, *Brassica oleracea* (cabbage), and *Syringa vulgaris* has been determined by means of thermocouples under various environmental conditions, but mostly in bright sunlight in the open and in the greenhouse. These leaves were nearly always warmer than the air during the day, and in direct sunlight they were frequently between 5° and 10° C. warmer. The maximum difference between leaf and air temperatures recorded in the open was 13.1° C., and in the greenhouse, 16° C.

2. Transpiration rates were measured while the temperature determinations were being made, by loss of weight from potted plants and by Ganong potometers. Transpiration was checked in certain plants by allowing the soil to become dry, and in some leaves by vaselining the surface. In general, the plants in dry soil and the vaselined leaves were 2° to 4° C. warmer than the controls. A similar difference in temperature was observed between fresh green leaves and pressed dried ones. But in no case was a definite correlation found between the transpiration rate and the difference between the leaf and air temperatures, nor between the difference of the transpiration rates of two leaves or plants, and the difference of their temperatures.

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## THE EFFECT OF TRANSPIRATION AND ENVIRONMENTAL FACTORS ON LEAF TEMPERATURES II. LIGHT IN- TENSITY AND THE RELATION OF TRANSPI- RATION TO THE THERMAL DEATH POINT

HAROLD H. CLUM

(Received for publication May 22, 1925)

In a previous paper <sup>1</sup> it was shown that the cooling effect of transpiration upon the leaves of certain common plants is seldom more than 2° or 3° C. In this paper data are presented to show the effect upon leaf temperatures of a slight inequality in the illumination upon two sides of a leaf, and the effect of shading a leaf from the direct rays of the sun. An attempt was also made to determine the thermal death point of the plants studied, and to see if transpiration does actually protect them from injury in bright sunlight. Thermocouples were used in these experiments as described in the previous paper.

In most of the experiments reported in these papers two junctions, one in the middle of the lamina on each side of the midrib, were placed in each leaf under observation in order to obtain a better idea of the temperature of the whole leaf. Sometimes the readings from these two junctions in the same leaf varied quite markedly. A striking example of this sort is shown in the following experiment with cabbage leaves in potometers. Table 1 gives the temperature readings of the two sides of each leaf, the air temperatures, the differences between successive readings on the two sides of each leaf, and the differences between the means of the readings from each side of the leaves. At each time indicated at the left, all the junctions were read as rapidly as possible, and the two readings from the same leaf were always observed as quickly as the instrument could be operated and were recorded after the second determination had been made. The sun was bright but the atmosphere was hazy, and during the afternoon there were gusty winds which tended to make all the temperatures fluctuate. Both leaves were somewhat curved on the edges, and the junctions had been

<sup>1</sup> Clum, H. H. The effect of transpiration and environmental factors on leaf temperatures I. Transpiration. Amer. Jour. Bot. 13: 194-216. 1926.

[The Journal for March (13: 167-216) was issued March 11, 1926.]

TABLE I. *The Variation in Temperature of Opposite Sides of the Same Cabbage Leaf, June 21, 1923 (Weather Bright, Hazy, Gusty Wind)*

Time A.M.	Leaf A				Leaf B			Diff., A-B	Air Temp.	R. H.
	Junc. 1	Junc. 2	Diff., 1-2	Mean, 1 and 2	Junc. 3	Junc. 4	Diff., 3-4	Mean, 3 and 4		
11:20	35.62°	36.12°	- 0.5°	35.87°	36.04°	35.82°	+ 0.22°	35.93°	31.5°	
26	35.97	38.00	- 2.03	36.98	36.89	36.57	+ 0.32	36.73	32.3	
40	36.87	38.73	- 1.86	37.8	35.95	35.17	+ 0.78	35.56	30.5	48%
47	37.11	37.03	+ 0.08	37.07	35.44	33.9	+ 1.54	34.67	31.2	46%
58	35.91	37.3	- 1.39	36.6	37.26	36.67	+ 0.59	36.96	33.6	
12:04	35.82	37.8	- 1.98	36.81	37.96	36.55	+ 1.41	37.25	34.2	
Means Probable Errors	36.22°	37.5°	- 1.28° ± 0.2208	36.85°	36.65°	35.78°	+ 0.81° ± 0.1391	36.18°	32.22° <sub>2</sub>	47%
P.M.	Vasclined Leaf A									
12:34	37.54°	37.00°	+ 0.54°	37.27°	32.85°	33.25°	- 0.4°	33.05°	32.0°	45%
50	40.12	40.12	0.0	40.12	36.68	34.8	+ 1.88	37.25	34.9	43%
2:00	38.63	37.97	+ 0.66	38.3	35.64	34.86	+ 0.78	35.25	31.2	
3:26	38.7	36.59	+ 2.11	37.64	35.37	33.83	+ 1.54	34.6	33.2	
31	41.0	35.73	+ 5.27	38.36	35.98	33.43	+ 1.55	34.2	32.1	45%
43	42.53	37.2	+ 5.33	39.86	36.33	33.13	+ 3.2	34.73	32.8	
					35.77	32.8	+ 3.97	34.78		
	41.03	36.42	+ 4.61	38.72	37.13	33.5	+ 3.63	35.31	33.6	
58	40.4	35.5	+ 4.9	37.95	36.42	32.97	+ 3.45	34.69	33.2	
4:04	40.9	38.22	+ 2.68	39.56	36.33	33.03	+ 3.3	34.68	34.2	42%
14	40.85	36.43	+ 4.42	38.64	35.1	33.18	+ 1.92	34.14	33.0	43%
	38.89	35.08	+ 3.81	36.98	35.4	31.99	+ 3.41	33.69	30.3	
Means Probable Errors	40.05°	36.93°	+ 3.12° ± 0.3913	38.49°	35.75°	33.4°	+ 2.35° ± 0.2523	34.7°	32.77°	44%

placed about in the middle of the right and left sides of each leaf. Both leaves stood in the potometers in such a position that the sun's rays struck the middle of the leaf at as nearly a right angle as possible, and they were adjusted several times to try to correct these angles. In spite of all that could be done, the differences between the readings from the opposite sides of the same leaf were over  $1^{\circ}$  C. in two thirds of the cases, and sometimes went as high as  $4^{\circ}$  or  $5^{\circ}$  C. It seemed that nothing could account for these differences except the slight difference in the angle at which the sun's rays struck the two sides of the leaf. The temperature might have changed between readings, but, if that were the only cause of the discrepancies, one side of the leaf would not appear as constantly warmer than the other as is shown in the table.

It will be noticed that, before vaselining leaf *A*, the mean of the differences between the individual temperature readings of the two leaves was less than that between the two sides of either leaf, and that this difference was not significant as shown by the large probable error; while the differences between the temperatures of the opposite sides of the same leaf were significant for both leaves. After vaselining leaf *A*, however, this leaf was sufficiently warmer than leaf *B* to give a significant difference in spite of the wide variations in temperature. The experiment shows that different parts of the same leaf may vary in temperature, in consequence of a difference in the angle at which the sun's rays strike the leaf or of some other factor; and that this variation may be nearly as great as the difference in temperature caused by checking transpiration by vaselining one of the leaves.

#### THE FLUCTUATION OF LEAF TEMPERATURE WITH ALTERNATE SHADING AND EXPOSURE TO SUNLIGHT

In most of the experiments reported in this and in the previous paper, the fact that leaf temperatures are subject to very sudden and wide fluctuations has been repeatedly noted. Some of these fluctuations are apparently due to changing air currents and convection, but the most pronounced ones are due to variations in light intensity. Similar observations on the fluctuations of leaf temperatures have been made by Blackman and Matthaei (1), Smith (12), Ehlers (2); and Harvey (3, 4) has shown nearly as great variations in the temperature of the cambium of woody stems.

These variations in temperature were noticed by the present writer before much work had been done, and in order to follow them an experiment was performed with *Fuchsia* plants in the summer of 1922. The leaves containing the junctions were shaded at intervals from the direct rays of the sun by a small board placed about a foot in front of the leaves. After shading, readings of the temperature of one leaf were taken as rapidly as possible until the temperature became quite constant. Then the shade was removed and the temperature again followed. The first reading after

placing or removing the board was usually made in 30 or 40 seconds. In that time the temperature of the leaf frequently changed  $4^{\circ}$  or  $5^{\circ}$  C. from that read about a half minute before moving the shade. At one time a rise of  $8^{\circ}$  C. was observed in 35 seconds.

Only one such experiment was tried that summer, but during the winter similar experiments were performed in the greenhouse, and in the summer of 1923 several experiments were performed out of doors with a variety of plants. The shade was put in place and removed several times during each experiment, and the temperatures of a normal, of a vaselined, and of a pressed dried leaf were all followed. The curves of the temperatures of all the leaves were very similar, but to save repetition, only one shading from each of four experiments is shown in the accompanying graphs. Changes of three or four degrees as the result of shading or exposure to sunlight were very common. The graphs show the actual data of some of the more striking instances. In making these graphs and in estimating the sudden fall and rise in leaf temperature, it was assumed that the leaf temperature remained constant from the time of the last reading until the shade was changed. This probably was not true within some degrees if much time had elapsed, but the temperature of the leaf was always read a few seconds before the shade was changed, and so the error could not have been large.

Text figure 1 shows the temperature changes of a *Fuchsia* leaf. At 3:32 P.M. the temperature of the air was  $28.8^{\circ}$  C., while that of the leaf was  $34.35^{\circ}$ , or  $5.55^{\circ}$  above the air temperature. A half minute later the shade was put in place, and in 20 seconds the leaf temperature had fallen to  $31^{\circ}$ , a drop of  $3.35^{\circ}$  C. It will be noticed that the air temperature reading was  $29.4^{\circ}$ , which was higher than before the shade was put in place. It would seem, therefore, that shading had little effect upon the air temperature. This was to be expected, especially since the junction by which the air temperature was determined was always kept in the shade of one of the leaves. After the shade had been on for ten minutes, the air and leaf temperatures were again determined and the shade was quickly removed. In 15 seconds the leaf temperature rose from  $27.71^{\circ}$  to  $34.81^{\circ}$ , a rise of  $7.1^{\circ}$  C.

Text figure 2 shows the effect of shading upon the temperature of lilac leaves. In this case the temperatures of a normal green leaf, leaf *B*, in a potometer, and that of a pressed dried leaf, leaf *F*, were followed simultaneously. Exposed to the sun, the temperature of leaf *B* was  $38.87^{\circ}$  and that of leaf *F* was  $39.32^{\circ}$ , while the air temperature was  $29.6^{\circ}$  C. Twenty-three seconds after shading, the temperature of leaf *B* had dropped  $5.89^{\circ}$  and that of leaf *F*  $6.34^{\circ}$ , so that both had the same temperature,  $32.98^{\circ}$ . The next readings, taken 42 seconds later, also showed the leaves to be at nearly the same temperature, and after that leaf *B* was only about one degree cooler. Just before removing the shade both leaves were again at the same temperature,  $31.44^{\circ}$ , and this was  $0.16^{\circ}$  cooler than the air temper-

ature. In 35 seconds after removing the shade, leaf *B* rose  $5.59^{\circ}$  while leaf *F* rose  $7.56^{\circ}$ . The readings were then  $37.03^{\circ}$  and  $39.0^{\circ}$  respectively. In the next 45 seconds, however, leaf *B* rose to  $40.98^{\circ}$  while leaf *F* rose only to

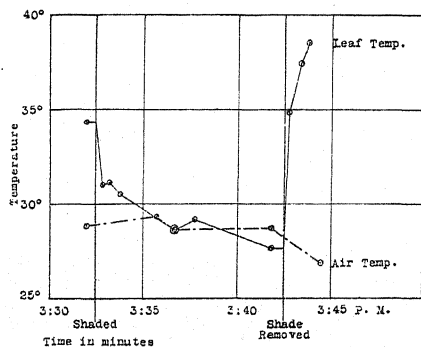


Figure 1

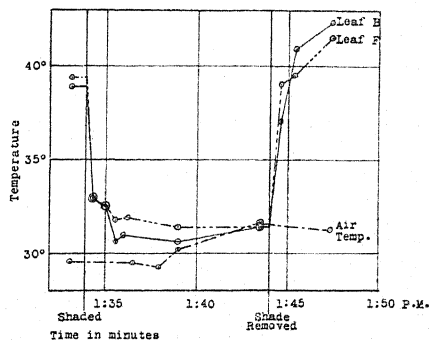


Figure 2

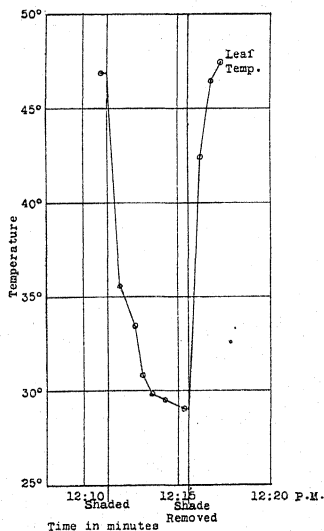


Figure 3

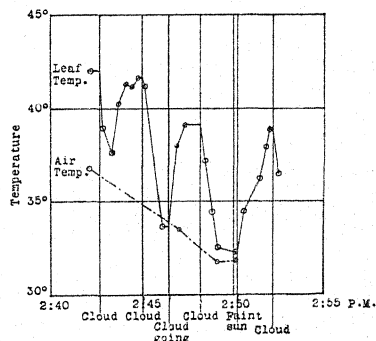


Figure 4

Curves showing the effects of sudden shading and exposure to sunlight upon the temperatures of various leaves. TEXT FIG. 1. Temperature of a Fuchsia leaf. July 12, 1923. TEXT FIG. 2. Temperatures of lilac leaves; leaf *B*, a normal green leaf; leaf *F*, dried. July 14, 1923. TEXT FIG. 3. Temperature of a lettuce leaf. Experiment performed in the greenhouse, January 30, 1923. TEXT FIG. 4. Temperature of a privet leaf. Shading caused by passing clouds. July 21, 1923.

$39.53^{\circ}$ , so that the normal leaf was  $1.45^{\circ}$  warmer than the dried one. From this experiment it is evident that the high water content of the normal leaf had little effect in stabilizing its temperature, since this leaf fluctuated about as rapidly as the dried leaf when shaded and exposed to the sun.



Text figure 3 shows the greatest fall and rise in leaf temperature observed by the writer. The material used was the "Big Boston" variety of lettuce, *Lactuca sativa* L., with which a few temperature experiments were performed in conjunction with Mr. A. G. Newhall of the Department of Plant Pathology. This experiment was performed in the greenhouse with the plants in direct sunlight on January 30, 1923. The plant had been taken out of the ground and was standing in water, but by the time the experiment with shading was performed it had begun to wilt. A thermocouple was placed in the midrib of an outer leaf fully exposed to the sun. The temperature of this leaf was  $46.93^{\circ}\text{C}$ . at 12:10 P.M. A shade which cut off only the direct rays of the sun was held about a foot from the plant. In 35 seconds after putting the shade in place the leaf temperature fell to  $35.52^{\circ}$ , a drop of  $11.41^{\circ}\text{C}$ . The temperature continued to fall, but more gradually, until it reached  $29.02^{\circ}\text{C}$ . This must have been only a few degrees above the air temperature, for although the latter was not determined at the time, it had been  $24.8^{\circ}\text{C}$ . about 15 minutes before. The shade was now removed, and in 35 seconds the leaf temperature jumped to  $42.45^{\circ}\text{C}$ ., a rise of  $13.43^{\circ}$ .

To determine whether the temperature of leaves of a plant growing normally would also fluctuate as greatly as that of leaves of a potted plant or of cut leaves already investigated, some experiments were performed with leaves of a privet plant, *Ligustrum ovalifolium*, growing just outside the greenhouse. The temperature of these leaves fluctuated in a manner very similar to that of the other plants, both when artificially shaded and when shaded by passing clouds. Text figure 4 shows some of the temperature changes due to the shade of passing clouds. In one case the leaf temperature fell seven degrees in less than a minute.

These experiments of following the rapid fluctuations of leaf temperature show very strikingly that the leaf temperature is not at all a stable thing, and that in the light it does not approximate that of the air except within a wide range. It is dependent primarily upon the amount of light energy which the leaf can absorb. Because of its large flat surface in proportion to its other dimensions, the leaf will become heated in strong sunlight and cooler in the shade so rapidly that the presence of a large percentage of water seems to make little difference; a fresh leaf will fluctuate as rapidly, and over as wide a range, as a dried one. The changes in temperature due to differences in light intensity are so much greater than the differences due to transpiration that the latter seem to be insignificant. Since a leaf, whether it is transpiring or not, can lose energy rapidly enough to lower the temperature of the whole five or six degrees in half a minute when shaded from the direct rays of the sun, and since the cooling effect of transpiration is usually only two or three degrees, it would seem that radiation and convection from the leaf must be far more important than transpiration in keeping the leaf from becoming hotter than it does in bright sunlight. Transpiration plays a part, no doubt, but only a very small part.

## THE THERMAL DEATH POINT OF PLANTS

The cooling effect of transpiration has been shown to be very small. To determine whether this small temperature difference might be of benefit to the plant under intense insolation, it was necessary to know the thermal death point of the plants studied. This point has been determined for certain plants by other workers. Sachs (11) found that the vegetative parts of a number of terrestrial plants could survive a temperature of 50° or 51° C. for 15 minutes without injury, but were killed if heated between 51° and 52° C. for 10 minutes. Aquatic plants in water were injured by an exposure to a temperature of only 45° or 46° C. for 10 minutes. Mayr (7) and Münch (8, 9, 10) both state that plant tissues are killed at about 54° C. and attribute an injury, noticed on tree seedlings in the nursery at the point where the stem comes in contact with the soil, to burning, since cases are reported in which the surface layer of soil was at a temperature of 62° and even of 68° C. in bright sunlight. Tubeuf (13) says that Hartig found the cambium killed between 52° and 55° C., and that De Vries found corn, beans, Iris, and Vinca killed between 47° and 55° C. Leitch (5) has shown the thermal death point of roots of *Pisum sativum* to be below 45° C. The thermal death point of many plants, therefore, lies between 45° and 55° C., depending upon the nature of the plant, the conditions within the plant, and the duration of the exposure to the high temperature.

To determine more accurately, however, the thermal death point of the plants studied, and to see whether the ability to transpire would prolong the life of the plant under extreme conditions, the following experiments were performed. In no case observed by the writer were the leaves of a plant in the open injured by bright sunlight, but it was found that plants which had been placed in a glass chamber to change their transpiration rates by varying the relative humidity were sometimes scorched. The glass chambers were then used to heat the leaves abnormally in determining the thermal death point, and the browning of the leaves was taken as an indication of injury.

Table 2 gives the results of an experiment which is typical of the experiments with Fuchsia plants. Six potted plants were used. One in dry soil and another in wet soil were placed in a glass chamber in a humid atmosphere. The humidity was maintained with wet sphagnum. Another pair of plants, one in wet soil and the other in dry soil, was placed in another glass chamber, the humidity of which was kept low with calcium chlorid. A similar pair of plants was set in the open beside the glass chambers. Leaves of each plant with a similar exposure to the sun were selected for inserting the junctions. The temperature of the plants in dry soil was much higher than that of the plants in wet soil throughout most of the experiment. The plant in dry soil in the dry chamber was the first to show injury due to overheating, but the first injured leaf was not the one with the junction in it. The exact temperature at which injury occurred, therefore, was not

TABLE 2. *The Thermal Death Point of Fuchsia Plants, August 26, 1922*

Time A.M.	9:33	9:50	10:00	10:17	10:35	11:00	12:30 P.M.	12:45	Transp. G. Sq. Dec. Hr.
In Moist Chamber:									
Plant 1, Wet soil.....	38.6°	36.8°	40.4°	41.1°	41.6°	43.8°	49.8°*	47.6°	1.060
Diff., 1-3, 1-air.....	+3.7	+1.6	+1.9	+0.4	+0.1	-0.9	-0.6	+0.1	
Plant 2, Dry soil.....	45.5	43.7	47.1*	49.5	48.3	53.0†	60.8	54.8	0.630
Diff., 2-3, 2-air.....	+16.6	+12.5	+8.6	+8.8	+6.8	+8.3	+10.4	+7.3	
3, Air temp. ....	34.9	35.2	38.5	40.7	41.5	44.7	50.4	47.5	
In Dry Chamber:									
Plant 4, Wet soil.....	39.5°	44.3°	45.3°	45.8°	45.8°	48.0°†			2.70
Diff., 4-6, 4-air.....	-1.5	-0.5	-0.3	-0.4	-1.5	+0.8			
Plant 5, Dry soil.....	49.3	49.2*	47.9	50.6†	52.0	53.3			0.880
Diff., 5-6, 5-air.....	+8.3	+4.4	+2.3	+4.4	+4.7	+6.1			
6, Air temp. ....	41.0	44.8	45.6	46.2	47.3	47.2			
In the Open:									
Plant 7, Wet soil.....	29.8°	34.6°	35.1°	33.6°	34.2°	34.9°	33.5°	33.9°	0.748
Diff., 7-9, 7-air.....	+5.5	+7.0	+5.1	+6.1	+7.1	+6.4	+6.0	+5.2	
Plant 8, Dry soil.....	32.9	37.1	39.3	38.1	36.7	36.2	33.0	32.8	0.354
Diff., 8-9, 8-air.....	+8.6	+9.5	+9.3	+10.6	+9.6	+7.7	+5.5	+4.1	
9, Air temp. ....	24.3	27.6	30.0	27.5	27.1	28.5	27.5	28.7	

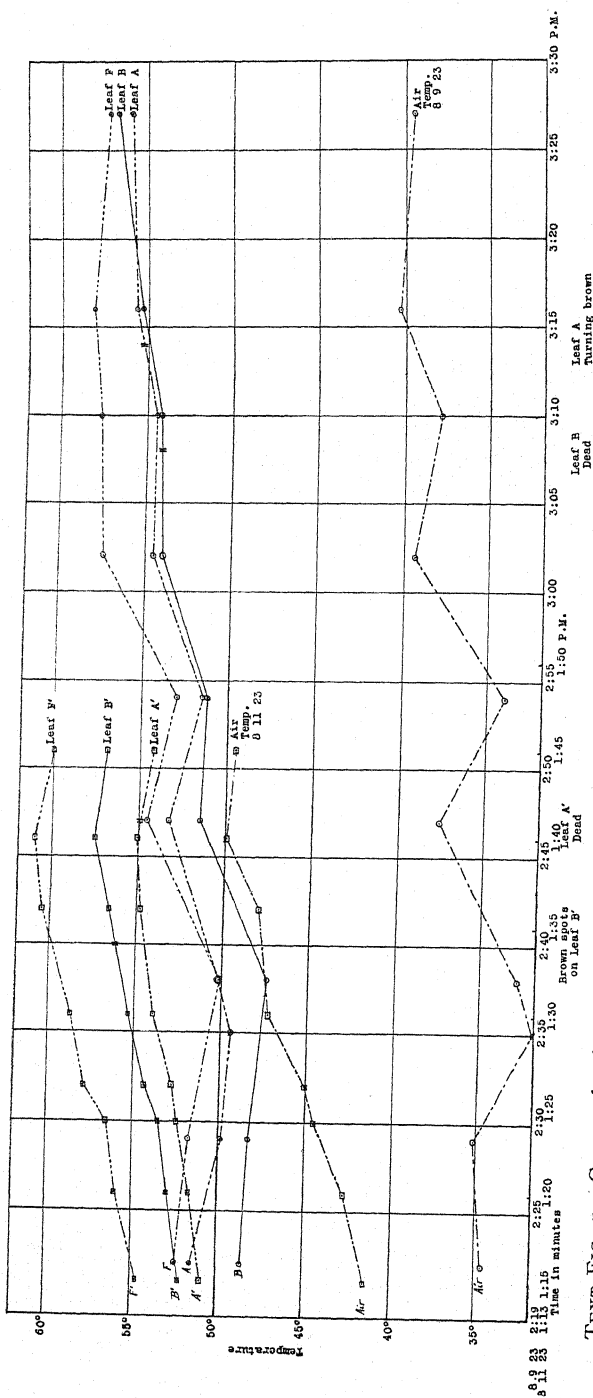
\* Plant injured.

† Leaf dead.

certain. This plant had a higher temperature than the plants in the moist chamber, but the temperature of the air around it was also higher. This may have been because the drops of water which condensed on the walls of the moist chamber cut down to some extent the intensity of the sunlight in that chamber. The temperatures of the leaves in the moist chamber were further above the temperature of the air than were the temperatures of those in the dry chamber. Part of this difference might have been due to a slower transpiration rate. The most rapid transpiration was exhibited by the plant in wet soil in the dry chamber, and this plant was actually cooler than the air throughout most of the experiment. In both chambers the plants in wet soil lasted about an hour longer than the plants in dry soil. In all cases injury was observed when the leaves had reached a temperature between  $48^{\circ}$  and  $50^{\circ}$  C. The difference between the air temperature and the temperature of the leaves of the plant in dry soil outside was not quite as great as the difference between the leaf temperature of the plant in dry soil in the moist chamber and the temperature of the air around it, and yet the plant outside was transpiring much more slowly. This result showed that, when the plants were under normal conditions, exposed to variable air currents and not confined by glass walls, radiation and convection had a considerable cooling effect upon the leaves, because they retained less heat although transpiring more slowly. In the moist chamber the leaves were heated more highly in spite of a high transpiration rate. The high temperature may have been partly responsible for the more rapid transpiration. Under these abnormal conditions, when the air and convection currents were confined and the heat waves radiating from the leaves were also confined, the cooling effect of transpiration became more noticeable. The differences in temperature between the plants in wet and in dry soil were greater in the chambers than outside. Under such circumstances it might be said that transpiration protected the leaves of the plants in wet soil from overheating and burning.

In this and in other experiments with *Fuchsia* plants it was found that the leaves were injured between  $48^{\circ}$  and  $50^{\circ}$  C., dying more quickly if the temperature was high. But an exact thermal death point can not be given, because, as pointed out by Lepeschkin (6), the duration of a given temperature is important and must be given, and in these experiments no precautions were taken to keep the temperatures constant until the plant died.

On August 9, 1923, an experiment with lilac leaves in potometers was performed to determine the thermal death point of these leaves and to study the possible beneficial cooling effects of transpiration. The leaves were placed in the potometers and the thermocouples inserted as usual. One of the leaves was vaselined on the under surface, and a dried leaf was placed beside the two potometers. After some preliminary readings the glass chamber was lowered over the leaves. Transpiration rates could not be determined from the potometers, however, because opening the chambers



TEXT FIG. 5. Curves showing the temperatures of lilac leaves in a glass chamber in the sunlight in two different experiments. First experiment, August 9, 1923: leaf *A*, vaselined; leaf *B*, not vaselined; leaf *F*, dried. Second experiment, August 11, 1923: leaf *A'* vaselined, leaf *B'* not vaselined, leaf *F'* dried. The times at which the leaves became injured are indicated on the curves and at the bottom.

to reset the potometers every few minutes would spoil the heating effects of confining the air; and furthermore, as the temperature became very high, the grafting wax about the petioles of the leaves melted and the potometers leaked. In spite of the leakage, however, the leaves did not lack for water throughout the experiment.

The results of this experiment and of one performed two days later are shown graphically in text figure 5. Temperatures are plotted on the ordinates and time in minutes on the abscissae. The upper row of figures at the bottom of the graph represents the exact times at which temperature readings were taken during the first experiment, and the lower row represents the same for the second experiment. The graphs have been so arranged that the time of covering the leaves with the glass chamber falls on the same line for both experiments. This was at 2:19 P.M. in the first experiment, and 1:13 P.M. in the second. Only the data taken after placing the glass chamber are shown in the graph.

Before the glass chamber was placed over the leaves there was very little difference between the temperatures of the normal, the vaselined, and the dried leaves. In fact, the dried leaf was sometimes cooler than the others. Three minutes after placing the chamber over them, the temperature of the dried leaf was  $52.4^{\circ}$  C. in the first experiment, that of the vaselined leaf  $51.6^{\circ}$ , and that of the unvaselined only  $48.7^{\circ}$  C. For 25 minutes the unvaselined leaf remained below  $50^{\circ}$  C. Twenty-one minutes after the first temperature reading over  $50^{\circ}$  had been taken on this leaf, it died. This was 24 minutes after the line representing the temperature of the leaf had gone above  $50^{\circ}$  C., as shown on the graph. The vaselined leaf did not turn brown, which turning was taken as a criterion of injury, until 6 minutes later.

The experiment performed on August 11 was run in the same manner as the one just described. The leaf temperatures, and especially the air temperature, were higher than in the former experiment. Two minutes after placing the glass chamber the temperatures of all the leaves were over  $50^{\circ}$  C.: that of the dried leaf  $54.7^{\circ}$ , that of the vaselined leaf  $50.9^{\circ}$ , and that of the unvaselined leaf  $52.2^{\circ}$ . In 21 minutes the unvaselined leaf had two brown spots, and the vaselined leaf appeared bleached and withered. Two minutes later half of the unvaselined leaf was brown and dead. After a few minutes more the vaselined leaf was evidently dead, but it did not turn brown as rapidly as did the normal leaf. It was thought that the reason the vaselined leaves turned brown so slowly in these experiments was that the vaseline blocked the stomata and prevented rapid oxidation of the dead tissue. It may have been that the vaselined leaves actually died first, but their appearance did not change sufficiently to indicate that they had died.

The striking fact about these experiments was that in both cases the unvaselined leaf died in 20 to 25 minutes after its temperature had gone above  $50^{\circ}$  C. During the first 25 minutes after the glass chamber had been

put in place in the former experiment, the temperatures of the dried and vaselined leaves were above  $50^{\circ}\text{C}.$ , or very close to that, while the temperature of the normal leaf was one or more degrees below. In that interval it might be said that the higher transpiration rate of the normal leaf protected it from burning, since the other leaves which could not transpire were at temperatures high enough to kill them. These differences in temperature may have been due to differences in transpiration. The thermal death point of lilac leaves is probably close to  $50^{\circ}\text{C}.$

Experiments were also performed with young cabbage plants and with the leaves of a privet plant growing outside the greenhouse, to determine their thermal death points. The experiments were not altogether successful, however. The young cabbages were difficult to heat to  $50^{\circ}\text{C}.$  even in the glass chamber, and little injury resulted. The privet leaves were very resistant. They withstood a temperature of over  $50^{\circ}\text{C}.$  for 40 minutes without injury.

#### DISCUSSION

From the experiments reported in these papers, it is evident that transpiration may cool the leaves of a plant  $2^{\circ}$  or  $3^{\circ}\text{C}.$  and in some cases a little more, but this reduction in temperature seems insignificant when compared with the effect of other factors as shown in this paper. The intensity of the light is probably the most important factor, and with this is coupled the angle at which the sun's rays strike the leaf. It has been shown that shading a leaf from the direct rays of the sun may lower its temperature  $5^{\circ}$  to  $7^{\circ}\text{C}.$  or more in less than a minute, and that exposing it again to the sun may have as great an effect in the opposite direction. Changing the angle of the leaf to the rays of light may cause a change in the temperature as great as could be produced by any possible change in transpiration rate. Since the leaf will cool so quickly when shaded, it is evident that it must lose heat very rapidly by radiation and convection. This is probably an important factor, and may be one reason why a dried leaf is so little warmer than a green one. Convection is augmented by air currents. In nature the majority of leaves are not at right angles to the sun's rays, and thus can not be heated to the maximum. Those which are most exposed probably are kept cool more by convection and radiation than by transpiration, especially when the air is moving, as is usually the case. The leaves of the plants studied were not injured until after their temperature had remained near  $50^{\circ}\text{C}.$  for some time. Although the summer of 1923 was not as hot as it might have been, there were some hot days; and from the data obtained, it seems that ordinarily, in a climate such as that of Ithaca, New York, the sun will not heat leaves to  $50^{\circ}\text{C}.$ , even when transpiration is checked. Only under the most extreme conditions, when leaves would be heated above their thermal death point were it not for transpiration cooling them a few degrees under that point, can it be said that transpiration really protects them. Even in such a situation, they would soon die at the cooler temper-

ature, if this remained constant for any length of time. But the temperature does not remain constant. Under such conditions, the rapid radiation and convection, together with varying air currents, cause a constant fluctuation of temperature, and are probably more effective in protecting the leaves from overheating than is transpiration.

#### SUMMARY

1. Very rapid and wide fluctuations of leaf temperature were noticed in *Fuchsia speciosa*, *Brassica oleracea* (cabbage), *Syringa vulgaris*, *Lactuca sativa*, and *Ligustrum ovalifolium*. The effect was especially great when the leaves were suddenly shaded and exposed to the sun, either artificially or by a passing cloud. A change of 5° to 7° C. in less than a minute was quite common. Fresh green leaves varied as much as pressed dried ones. The maximum fluctuation was observed in the greenhouse, using a fresh lettuce leaf, a rise of 13.4° C. in 35 seconds. The angle at which the sun's rays struck the leaf also had a marked effect upon its temperature.

2. An attempt was made to determine the thermal death point of the plants studied, by allowing the temperature to rise abnormally high in an enclosed glass chamber in sunlight. The leaves of *Fuchsia* and of lilac were injured at about 50° C., but those of privet appeared to be more resistant. In the open the temperature of the leaves studied did not reach this point, even in bright sunlight in summer.

3. Since the cooling effect of transpiration is so small, as shown in a previous paper, compared with the much greater effects upon leaf temperature of such factors as light intensity, angle of incidence, convection, radiation, and air currents; and since the sun did not heat even a dried leaf to an injurious temperature in any experiment performed in the open; and since, under severe conditions, lowering the temperature by 2° or 3° C. would not greatly prolong the life of a plant if the temperature remained constant; it is thought that, in a climate such as that of Ithaca, New York, the cooling effect of transpiration is insignificant and never actually protects the leaves of the plants studied from burning in bright sunlight.

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## FURTHER CULTURAL LIFE HISTORIES OF THE STROMATIC SPHAERIALES<sup>1</sup>

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In the following pages are given the life histories of some stromatic Sphaeriales which have produced perithecial stromata in culture. These life histories have been obtained in the course of a cultural study of these forms in an attempt to determine the developmental relationships within this group.

In a previous discussion of the occurrence of the perfect stage in culture (19), the writer has suggested that the ability to form perithecia under certain conditions seems to be characteristic of certain groups of closely related forms. The genera *Gnomonia*, *Glomerella*, and the related forms of the genus *Diaporthe*, for instance, seem to show unusual ability to form perithecia in agar cultures. In the paper just mentioned, perithecium-like bodies of *Diaporthe albovelata* (B. & C.) Sacc. were reported as occurring on oatmeal agar. Since that time a normal development of these bodies has been obtained on agar. These proved to be true perithecia and produced mature asci and spores. In the following discussion it is shown that *D. galericulata* (Tul.) Sacc. also produces perithecial stromata on oatmeal agar. Both these species belong to that group of the subgenus *Chorostate* which possesses appendiculate spores and lacks a blackened marginal line about the stroma.

Three species of the allantoid-spored group are also given which form perithecial initials on sterile twigs. Other species with allantoid spores have given immature perithecial stromata on twig cultures, but none of these forms have matured their perithecia under cultural conditions. This failure of these forms to produce ascospores is apparently due to the long period of development necessary for the maturing of the fruiting body, and the difficulty of maintaining favorable moisture relations in tube cultures over such an extended period.

The methods and media used in the isolation and culture of the following forms were the same as described in a previous paper (18).

### DIATRYPE STIGMA (HOFF.) DE NOT.

This has been a favorite species for study in this group. Under the name of *Stictosphaeria Hoffmanni* Tul. it was described and figured by the Tulasnes (17), and its development studied by Füsting (7); it is also discussed by

<sup>1</sup> Papers from the Department of Botany of the University of Michigan, no. 220.

Ruhland (15) in his study of these forms. It is our commonest effused Diatrype and is found on the bark of numerous hard woods. The periderm is thrown off early in its development, exposing widely erumpent stromatic crusts of a brownish or black color. The perithecia are elongated perpendicularly to the surface of the stroma, and are 300–500  $\mu$  long. They are crowded in a single row within a light-colored area of the upper bark, which is bounded below by a heavily darkened zone of tissue. Columns of this "entostromatic" tissue, as it is designated by Ruhland, extend in various places from this upper light-colored area, through the lower darkened and disintegrated bark tissue, to the wood surface. The perithecia are separately erumpent as flat, disc-like, quadrisulcate ostioles which do not project above the surface of the stroma. The asci are clavate, long-stipitate, and measure (p.sp.) 30–40 x 4–5  $\mu$ . The spores are biserial in the ascus, yellowish-hyaline, allantoid, 1-celled, and 6–8 x 2  $\mu$ .

The material for culture was obtained on *Acer saccharum* Marsh. near Ann Arbor, Michigan. Sprays of ascospores from this material were made on October 17, 1923. Twenty-four hours later the spores were greatly swollen (13 x 5  $\mu$ ), and had pushed out from 2 to 4 germ tubes 2–3  $\mu$  in diameter.

Single-spore cultures on 6-percent oatmeal agar showed the imperfect stage on November 6. The imperfect stage on agar was formed as small, superficial mycelial stromata composed of loosely woven, hyaline hyphae. These hyphal masses were 0.5–1 mm. in diameter, white at first but later of a yellowish tint when spore-formation began. The conidial masses were bright yellow to orange in color. These conidia were generally formed in locules, which were sometimes regular in shape with a definite hymenium, or at other times irregular and labyrinthiform without a definite hymenium. These locules often increased in size, became confluent, and broke open to the exterior, forming irregular exposed cavities bearing the hymenial tissue. The conidia (Pl. XII, fig. 2) were long-cylindrical, slightly curved, often taper-pointed at the ends, hyaline, 1-celled, and 9–12 x 1.5  $\mu$ . They were formed by the cutting off of the curved tips of short conidiophores, or as the side branches of the larger fertile hyphae.

On October 31, a sterile twig of *Acer saccharum* Marsh. was placed in a large test tube with its base immersed in 15–20 cc. of oatmeal agar, and inoculated from a single-spore culture. After growth had begun, the tube was placed at a temperature of 10° C. This culture later showed numerous spherical mycelial masses on the surface of the twig which produced conidia in their interior and resembled the conidial stromata formed on agar. On January 7, 1924, this culture was brought back to room temperature to see if any further development of fruiting bodies would take place. None appeared after seven days, and the culture was replaced in the cold room. On March 15, the culture was again brought to room temperature and moistened with a small amount of sterile water. Examination of the bark

tissues on March 17 showed that a thin, effused layer of hyaline, actively growing fungous tissue had developed just beneath the periderm. This layer was in general about  $25\ \mu$  in thickness, but at various points it became much thicker ( $250\text{--}300\ \mu$ ), and in these areas labyrinthiform conidial locules, containing the typical conidia, were formed (Pl. XII, fig. 1). This tissue is the "epistroma" of Füsting (7, p. 180), and will be discussed later. On March 21, three areas were found where the periderm had been ruptured and thrown back, exposing young perithecial stromata. These areas varied from 5 to 15 mm. in diameter; they were at first light tan in color, but soon became a dark brown. The surface was dotted with minute light-colored points, which, as we shall see, were the apices of the perithecial necks. On September 28, 1924, a twig of *Tilia americana* L. was inoculated from a single-spore culture. This culture was kept at room temperature, and six weeks later, on November 9, the first perithecial stroma was exposed. The bark tissues of this twig were so thoroughly disintegrated that no further development took place.

Füsting (7) has described the development of this species in detail, and his conclusions are in general similar to those arrived at by the writer from cultural material. The hyphae of the fungus penetrate throughout the bark tissues of the host, remaining, in the early stages of growth, chiefly within the cell walls. The walls of the bark cells are apparently gelatinized by the action of these hyphae, as they become swollen and distorted. Before stroma-formation begins these hyphae become more active in the upper bark layers. Numerous crystals (probably calcium oxalate), soluble in concentrated HCl, are seen at this time scattered throughout the bark tissue.

Füsting states that the stroma has the same extent as to area when first initiated, as when mature. Ruhland (15), on the other hand, says that growth starts at various points and progresses outward, giving a mosaic structure to the stroma. The first stromatic appearance of the fungus in culture is in the form of rather widely scattered conidial stromata. These stromata are cushions of the epistroma of Füsting, or of the ectostroma of Ruhland. There can be seen the beginnings of the effused portions of this tissue extending out from the margins of these ectostromatic cushions, just beneath the periderm. On *Tilia*, perithecial initials were found beneath these isolated conidial stromata. If growth does take place centrifugally from these points, as Ruhland claims, it is at first very rapid, and when the periderm is thrown off from the surface of the young stroma it is over rather wide areas, as previously mentioned. That a peripheral extension of this young stroma later takes place, however, is apparent from an examination of its margin. In Plate XII, figure 1, an attempt has been made to show the various stages of development exhibited in this marginal region. The mosaic structure described by Ruhland depends upon whether or not separate stromata are initiated in close enough proximity to one another to become confluent.

The ectostromatic layer originates from the hyphae which are proliferating in the upper layers of the bark cells. It first appears as a loosely woven mycelium, which develops in the region of the phellogen. This mycelium increases in amount, forming a thin stromatic layer. Meanwhile the perithecial initials are being produced in the form of spherical interwoven knots of hyphae; these come from the hyphae which are proliferating in the upper bark tissues. This increase of the hyphal elements in the upper bark layers is the beginning of the entostroma or bark-inhabiting stroma of Ruhland, or the hypostroma of Füsting. All of this development goes on beneath the periderm, before it is ruptured.

About the time the perithecial initials appear, the ectostroma undergoes a change. The cells of its hyphae increase enormously in size, and eventually form a palisade-like pseudoparenchymatous tissue, the cells of which measure  $15-40 \times 10-13 \mu$ . This enormous swelling of the ectostromatic tissue bursts apart the periderm, throwing back the cork cells above, which carry the ectostromatic palisade layer with them. This exposes the phellogen layers beneath. There immediately occurs a blackening of this tissue, as mentioned by Füsting. Both the phellogen cells and the hyphae which penetrate among them become blackened and form the rapidly darkening exposed crust of the entostroma. The perithecial initials, which arise just before the bursting of the periderm, send hyphae upward through the covering phellogen layers and begin their dissolution. These plug-like areas of hyaline hyphae formed above the perithecia, as a result of this growth, are not blackened like the rest of the ectostromatic surface, and the result is the scattered light-colored dots on the surface of the stroma previously mentioned.

At the time of the rupture of the periderm, the bark tissues are rather evenly disintegrated, filled with calcium oxalate crystals, and penetrated by fine hyaline hyphae. Shortly afterward, however, there appear narrow, blackened, outlining zones of tissue which cut off the upper bark tissues from the lower, with occasional enclosed columns of tissue extending to the wood. This blackened tissue is composed of dark-walled hyphae, which blacken and more or less fuse with the bark cells. Within the areas of upper bark cut off by this zone there now occurs, particularly about the developing perithecia, a further proliferation of hyphae which binds together the partially disintegrated bark elements into a compact stromatic mass, the entostroma. Without, *i.e.*, below, this area the bark tissues are further disintegrated and blackened, becoming a crumbling mass.

The perithecial initials contain a central deeply staining coil of "Woronin hyphae". The origin and development of this coil of hyphae was not followed. These hyphae contain numerous deeply staining granules and large nuclei; the hyphae increase in number and decrease in diameter with the development of the perithecial initial, and apparently form a meristematic tissue lining the perithecial cavity from which the asci, paraphyses, and periphyses arise.

The formation, in this species, of a well developed and deciduous ectostroma, which functions as a mechanical tissue for the throwing off of the periderm, is characteristic of both the effused and the isolated forms of the genus *Diatrype*, as already pointed out by Füssing (7), and Ruhland (15).

CRYPTOSPHAERIA POPULINA (PERS.) SACC.

This species shows a type of effused stromatic development different from that of *Diatrype stigma*. The stroma in this case does not throw off the periderm. The perithecia are separately erumpent through the periderm as minute, irregularly sulcate ostioles. The stroma is slightly raised above the surface of the bark. The perithecia are spherical or flattened, 500–600  $\mu$  in diameter, and imbedded in a light-colored area of bark cortex which is outlined by a darkened zone of tissue running parallel to the periderm, or occasionally dipping to the wood surface, enclosing bridges of this light-colored area. The bark tissues outside this darkened zone are strongly blackened and disintegrated. The asci are clavate, long-stipitate, and (p. sp.) 30–52  $\times$  5–8  $\mu$ . The spores are biserial, yellowish-hyaline, allantoid, 1-celled, and 8–10  $\times$  2  $\mu$ .

Material of this fungus was collected on *Populus tremuloides* Michx. near Ann Arbor. Sprays of ascospores were made on January 17, 1924. After 24 hours the spores had swollen to a size of 11–12  $\times$  4–5  $\mu$ , and had thrown out a short germ tube, 4–5  $\mu$  in diameter, from each end of the spore.

Cultures from a single ascus, on oatmeal agar, formed an even, whitish to gray, superficial mycelial growth. So far no imperfect stage of this fungus has been obtained on either agar or twig cultures.

A twig of *Populus grandidentata* Michx. with its base immersed in oatmeal agar was inoculated from a single-ascus culture on January 13. This culture was kept at 3–10° C. for two months and then removed to room temperature. At room temperature growth increased and small tufts of mycelium appeared on the surface of the twig. This superficial mycelium increased rapidly and formed a thick, matted growth, the hyphae of which soon became blackened. On April 23 no fruiting bodies were found beneath this mat, but minute pustules were visible beneath the periderm in advance of this mycelial growth. On May 26 the bark tissues beneath this blackened mat were found to be thoroughly blackened and disintegrated, as were the surface layers of the wood. Vertical sections just beyond this surface growth of hyphae showed young perithecia.

The hyphae of the fungus grow throughout the bark and the upper layers of the wood. These hyphae are at first hyaline and partially disintegrate the bark elements, separating the cells from one another. Later on these hyphae become blackened and complete the disintegration of the bark tissues. In twig cultures which were kept moist and at room temperature, the hyphal growth was rapid and this disintegration was complete, the

entire bark and upper wood layers becoming blackened. Small light-colored areas remained here and there. These areas resembled the darkened areas in character, except that the hyphae within them were hyaline instead of dark-colored. From the preceding description it would seem that this blackening process, whatever its cause may be, is a process following the first disintegrating action of the hyphae. The question naturally arises as to why this blackening does not extend over certain areas, particularly over the entostromatic areas containing the perithecia. An examination of the tissues shows in the dark areas a growth of broad, darkened hyphae. On the border line between the blackened and light-colored areas there is produced a dense, blackened mass of mixed host and fungous tissue. Just within this densely blackened zone, and on the margin of the light-colored areas, there is nearly always found a zone of thickly woven hyaline hyphae. Apparently as the cause of the blackening, whatever it may be, advances into this area of fine, hyaline hyphae there is formed the dense black mass, which appears as the marginal zone of these areas. This blackened crust then seems to act as an efficient barrier to the blackening of the tissues within. This fact would explain the immunity of the entostromatic areas to the blackening action, for they are penetrated by such a fine, hyaline mycelium. It is interesting to note that in the moist cultures above mentioned the blackening of the tissues was so rapid that no entostromatic areas were delimited beneath the periderm. It would seem that, unless moisture and other conditions are favorable for the formation of a fine, hyaline entostromatic mycelium before a blackening of the tissues takes place, no fruiting bodies will be formed.

In this species there is no deciduous ectostroma formed as in *Diatrype stigma*. Instead, there arises immediately beneath the periderm a compact layer of fine, hyaline hyphae, some 35-70  $\mu$  in thickness (Pl. XIII, fig. 1). The walls of these hyphae are rapidly blackened and thickened, becoming more or less fused together and forming a sclerotium-like protecting crust on the surface of the entostroma. The bark tissues to a depth of 0.5-1 mm. beneath the periderm are strongly disintegrated by the preliminary growth of the fungus, but are thickly interwoven with a fine, hyaline mycelium, binding them together in a stromatic mass, the entostroma. This area is bounded by a zone of blackened tissue as previously described. The perithecial initials arise in this entostromatic area as small hyphal knots, just beneath the periderm, before the formation of the sub-epidermal sclerotial tissue mentioned. Hyphae grow out from the upper surface of these perithecial primordia and penetrate between the periderm cells, separating and finally absorbing them. These hyphae proliferate in this area and form the perithecial neck, which is not blackened like the surrounding sclerotial tissue. The perithecia have not thus far been brought to maturity in culture.

## CRYPTOSPHERA EUNOMIA (Fr.) GREV.

The name *Cryptosphaeria eunomia* is used here provisionally for this species, although it differs from the other species of *Cryptosphaeria* in its septate spores; its involved synonymy will be discussed later. Material of this species was collected on *Fraxinus americana* L. near Ann Arbor. The presence of the stroma is often scarcely visible externally. Well developed, mature stromata are, however, indicated by widely effused areas of slightly raised periderm. These areas are peppered with minute black dots, which are the erumpent, papillate, or disc-like ostioles, that are only occasionally sulcate. A vertical section in the stromatic area shows the upper half of the bark tissues to be yellowish-white in color, while the lower half is blackish or brownish. This upper light-colored area is sharply defined by a ventral blackened zone. The perithecia are scattered in a single layer in this upper stromatic portion of the bark; they are 480–550  $\mu$  in diameter, and have short, stout necks, and walls some 25  $\mu$  thick. The periderm is not ruptured, the ostioles penetrating it separately. There is a small area of blackened periderm tissue about each ostiole where it penetrates this tissue. The asci are clavate, long-stipitate, and measure (p. sp.) 80–100 x 13  $\mu$ . The spores are biserial, allantoid, hyaline, and 1-celled at first, becoming dark-brown, 1–4-celled, and straight cylindrical at maturity. The spores measure (16) 20–26 x 3–4  $\mu$ .

The conidial locules (Pl. XII, fig. 3), found on these ash twigs were imbedded along with the perithecia in the upper entostromatic areas. These locules arose within stromatic mycelial masses, which formed within the bark tissues. The locules were formed by the cutting off of conidia from the hyphae in the interior of these masses of mycelium, the entire stroma finally being used in the production of spores. There usually arose a number of these stromatic masses in proximity to one another, so that the mature conidial fruiting body consisted of a number of irregular locules or of a large labyrinthiform one produced by their coalescence. The conidia (Pl. XII, fig. 7) were hyaline, 1-celled, strongly curved, and measured 30–40 x 1  $\mu$ . This is the *Cytosporina fraxini* E. & E., which Ellis (3, p. 223) gives as the imperfect stage of *Endoxyla fraxini* E. & E. This, as von Höhnelt (11) states, is the same as *Cytosporina millepunctata* Sacc., which Saccardo (16, vol. 3, p. 602) gives as the imperfect stage of *Cryptosphaeria millepunctata* Grev.

The twigs upon which this fungus was found were placed in a damp chamber for 4 days, and sprays of ascospores were then made on November 9, 1923. Twenty-four hours later the spores were germinating. The spores were somewhat swollen and measured about 32 x 6.5  $\mu$ . Upon germination the cross walls were very apparent, and the spore wall was often constricted at the septa (Pl. XII, fig. 5). Two or three germ tubes were pushed out, one from each end, and often one from a central cell of the spore. The



central portion of each spore was filled with hyaline protoplasm, which was continuous with that of the germ tube. The periphery of each cell contained a yellowish-brown, oily substance. Brefeld (1) studied *Cryptosphaeria eunomia* in culture and found that the spores became 2-4-septate upon germination. He obtained free conidiophores on stromata, and conidial locules within these stromata.

A single-spore culture of December 6, on 6-percent oatmeal agar, produced a white mycelial growth on the surface of the agar. This culture, when examined on May 20, showed numerous minute, conical pustules and a few larger pulvinate stromata. These stromata were composed of a matted, hyaline superficial mycelium and contained conidial locules, which were 200-400  $\mu$  in diameter. There were usually a number of these locules, especially in the larger stromata. The locules were various in shape but rather regular in outline. They were outlined by a differentiated, wall-like zone of dark-brown hyphae. Within this zone of darkened hyphae, there was a hymenium of filiform conidiophores, 2  $\mu$  in diameter, which bore conidia identical with those found on the original material.

On January 22, 1924, a sterile twig of *Fraxinus americana* L. was inoculated from a single-spore culture. On this and on other twig cultures subsequently made, a white mycelial growth and erumpent stromata were produced. These conidial stromata were ectostromatic and originated as small mycelial masses within the periderm, between the outer cork cells and the inner phelloderm layers. Growth was rapid in the moist air of the culture tubes, and these stromata usually burst through the periderm and became erumpent-superficial. The conidial locules typical of this species were formed within them. Conidia identical with those already described, together with a second type (Pl. XII, fig. 6) which was shorter and less strongly curved, were found in these fruiting bodies. These shorter conidia measured 13-20  $\times$  1  $\mu$ . The spore masses were usually reddish-orange, but on the drier portions of the twigs they were sometimes whitish in color. The conidial locules were occasionally also formed in the bark tissues, as found on twigs in nature. In certain areas there was formed a richly developed stromatic mycelium within the upper bark tissue, but no perithecial initials were seen.

The synonymy of this species is such an excellent example of the confusion existing in the classification of this group that the writer is tempted to give a brief account of its history. The fungus was apparently first described by Fries (6, p. 377) as *Sphaeria eunomia* in 1823. In 1826, Greville (8, vol. 4, p. 201), not being aware of Fries' description, described the same fungus as *Cryptosphaeria millepunctata* Grev. Greville's conception of the genus *Cryptosphaeria* was very broad, including all those species of *Sphaeria* remaining beneath the periderm. In fact, the first species described, namely, *C. Taxi* (8, vol. 1, p. 13), was an imperfect form. Greville gave no spore measurements, but Nylander (14) says that the spores measure 8-12  $\mu$

in length. Winter (20, p. 694) gives this species as a synonym of *C. populina* (Pers.) Sacc., apparently on the basis of such measurements. Von Höhnelt (11), however, states that *C. millepunctata* is the same fungus as *C. eunomia* Fr., which seems more probable, as they both occur on ash. Saccardo (16, vol. 1, p. 182) apparently considers *C. millepunctata* as the type species. In 1867, Füssing (7, p. 196) placed *Sphaeria eunomia* Fr. in a new genus, *Melanoplaca*. In 1923, both von Höhnelt (11) and Keissler (12) published on the synonymy of this species. According to von Höhnelt, the fungus was again found by Otth in 1870 and placed by Nitschke in a new genus *Cladosphaeria*, as *C. eunomioides* (Otth) Nit. Ellis was the next worker to describe this fungus as a new species. In 1890 (3) he called it *Thyridaria fraxini* E. & E., stating that it had polysporous asci. Berlese later showed that the asci were 8-spored, and in 1892 Ellis (4, p. 521) placed it under *Endoxyla* as *E. fraxini* E. & E. In 1897 Lindau (13) made *Thyridaria* a subgenus of *Kalmusia*, and called the fungus *K. fraxini* (E. & E.) Lind. In 1898 Lambotte and Fautrey (Rev. Myc. 20: 58) described the genus *Cryptosphaeria* with this fungus as the type species, calling it *C. fraxini* Lamb. & Faut., saying that it resembles *Cryptosphaeria millepunctata* Grev. In 1906 Traverso showed that this species is identical with *Endoxyla fraxini*, and called it *Cryptosphaeria fraxini* (E. & E.) Lamb. & Faut. Von Höhnelt goes further and again complicates matters by claiming that this species may also occur on decorticated wood as an eutypoid form, or that upon disintegration of the bark the perithecia may remain as superficial fruiting bodies, in which condition it is described by Saccardo (16, vol. 9, p. 812) as *Trematosphaeria fraxini* Richon. He develops the synonymy of such wood-inhabiting forms, and considers *Endoxyla parallela* (Fr.) Fck., *Valsaria stellulata* Rom., *Sphaeria astroidea* Fr., and *Endoxylina stellulata* Rom. as the same fungus. He concludes that the genera *Eutypa* Tul., *Cladosphaeria* Nit., *Endoxyla* Fck., *Eutypopsis* Karst., *Endoxylina* Rom., *Cryptosphaeria* Lamb. & Faut., and *Cryptosphaeria* Grev. are not sufficiently well separated, and that *Cladosphaeria*, *Endoxyla*, *Endoxylina*, *Eutypopsis*, and *Cryptosphaeria* are completely identical. In 1915, von Höhnelt (9) claimed that *Endoxyla* is not an allantoid-spored genus, and placed it as a subgenus of *Anthostoma*. He later reversed this decision (11), however, and considered the spores allantoid.

This history is an example of the confusion arising from the indiscriminate formation of new species and genera before any clear understanding of the relationships within a group has been established.

This species differs from the two previously described effused species in the entire lack of an ectostromatic tissue on the surface of the perithecial stroma. It also shows the formation of conidial locules within the entostroma, which does not occur in *Diatrype stigma*. It is interesting to note that under moist cultural conditions this fungus will nevertheless form an ectostromatic conidial tissue within the periderm.

## EUTYPELLA CERVICULATA (Fr.) SACC.

This species was collected by the writer on *Alnus tenuifolia* Nutt. near Copeland, Idaho, and in the Medicine Bow Mountains, Wyoming, on *Alnus glutinosa* Gaertn. at Grand Rapids, Michigan, and on *Carpinus caroliniana* Walt. at Ann Arbor. The details of the stromatic characters vary somewhat, but this variation is due in large degree to the conditions of growth, although some of these differences may be correlated with the host upon which the fungus grows. The stromata are evenly scattered, sometimes confluent, usually circular in outline, 1-3 mm. in diameter, and erumpent as a dense circular cluster of large, cylindrical ostioles, with a long-conical, sulcate tip. This disc is 1-2 mm. in diameter. The length of the ostioles and the size of the stromata vary with the rapidity of growth. The perithecia are usually radially elongated,  $800 \times 450-600 \mu$ . They have walls some  $30 \mu$  in thickness, long, rather stout necks, and are arranged in a somewhat polystichous manner. The perithecia lie within a strongly differentiated area of the bark cortex which is lighter in color than the remaining bark tissues, on account of a richly developed mycelial tissue within the bark cortex. This stromatic area is sharply outlined by a thick, blackened zone of compacted, black, thick-walled fungous cells. This blackened zone extends 1-2 mm. into the wood, where it spreads out between the stromatic areas. The bark tissue between the stromatic areas is disintegrated by the absorbing action of the hyphae, but is not bound together by a stromatic mycelium, so that the cells often crumble and fall apart, leaving the stromata standing out as blackened spherical masses attached to the periderm by the cluster of ostioles. The asci are cylindrical-clavate, long-stipitate, often with the spores clustered in the lower portion, leaving an empty tip, and  $35-52 \times 3-4 \mu$ . The spores are biserial or irregularly uniserial, allantoid, 1-celled, yellow-hyaline, and measure  $6.5-8 \times 2-2.5 \mu$ . The spores of the form on *Carpinus* are smaller, measuring  $5-6.5 (7) \times 1.5-2 \mu$ .

This fungus is one of a number of species of *Eutypella* which form a closely related group. The form on *Carpinus* mentioned fits the descriptions of *Eutypella cerviculata* (Fr.) Sacc. The forms on *Alnus* seem to be intermediate between two species; *Eutypella angulosa* (Nit.) Sacc. on *Betula* is described as having large stromata (2-4 mm.) and similar spores ( $6-8 \times 1.5-2 \mu$ ), while *E. alnifraga* (Wahl.) Sacc. on *Alnus* is said to have smaller stromata (1.5 mm.) and larger spores ( $8-10 \times 1.5-2 \mu$ ). Winter (20, p. 701) gives *E. similis* (Karst.) Sacc. as a form similar to *E. alnifraga* but with smaller ( $5-6 \times 1 \mu$ ) spores. He also states that *E. angulosa* differs from *E. prunastri* (Pers.) Sacc. only in the type of ostiole; *E. prunastri* in turn being very closely related to *E. sorbi* (Alb. & Schw.) Sacc. De Thümen's specimen (Myc. Univ. 1069) of *E. sorbi* shows a form closely related to *E. cerviculata* but with a highly developed stroma, elongated ostioles, and very small

perithecia. None of the descriptions except those of *E. cerviculata* mention the dark marginal line, but this is present in *E. sorbi*. Ellis (5) gives *Diatrype megastoma* E. & E. (N. A. F. 1556), and *Eutypella alpina* E. & E. (N. A. F. 3331, 3436) as synonyms of *E. cerviculata*. Ellis' N. A. F. 1556 is typical of the form occurring on *Alnus tenuifolia*. The exsiccata of *E. alpina* are also similar except that the stromata are elliptical rather than circular, and are surrounded by a collar of the periderm. These are characters, however, arising in consequence of the configuration of the host bark. Authentic material of the other species of this group was not available, and since these closely related species need a critical examination, the forms grown in the present study are referred to *Eutypella cerviculata* (Fr.) Sacc.

On March 1, 1923, sprays of ascospores were made from twigs of *Alnus tenuifolia*. Six days later no germination had taken place. On March 16 a similar spray was made of an ascospore suspension in Leonian's nutrient solution,<sup>2</sup> and again no germination took place. On March 17 a portion of the *Alnus* bark containing perithecia was placed in a damp chamber, and three days later sprays of a suspension from this material in sterile distilled water were made. Twenty-four hours later the spores were swelling, and after 48 hours were germinating by means of germ tubes from both ends of the spore.

A single-ascus culture made on 6-percent oatmeal agar on March 27 showed orange-colored spore horns on April 25. Conidial formation was found to occur in numerous small locules formed beneath the surface of the agar. These locules possessed a slightly differentiated wall of hyphae running parallel to their periphery. The conidia were cut off from the tips of hyaline, filiform conidiophores, measuring  $15-20 \times 1 \mu$ . The conidia (Pl. XIII, fig. 4) were cylindrical or somewhat tapered at the ends, slightly curved or crescent-shaped, 1-celled, hyaline, and measured  $11-15 \times 1 \mu$ . The locules did not break through the surface at first, but appeared as numerous minute papillae. These locules soon became confluent, and the masses of spores then burst through the surface, forming large open pustules, 0.2-1 mm. in diameter.

On March 22 a suspension of ascospores was sprayed on to a sterile twig of *Alnus glutinosa*. Six to seven days later mycelium became visible upon this twig, and about May 1 spore horns appeared. On May 28 a twig of *A. glutinosa* was inoculated from a single-ascus culture. When examined on January 1, 1924, this twig showed both the imperfect stage and perithecial initials.

Both perfect and imperfect stromata were initiated by the formation of an ectostromatic mat of hyphae within and beneath the periderm. The hyphae of this mat penetrated the layers of periderm cells, where they proliferated and split these cells apart. In culture, these hyphae, together with a lenticular mat beneath the periderm, formed a rather large stromatic

<sup>2</sup> Amer. Jour. Bot. 11: 21. 1924.

plug, which soon became erumpent and produced a superficial growth of hyphae upon the twig. While this ectostroma was being formed, there was also a rapid growth of entostromatic hyphae within the bark tissues beneath. Flattened patches of stromatic mycelium spread out between the cell layers, and the entire host tissue was penetrated by a rich mycelium. Meanwhile a zone of small blackened pseudoparenchymatous cells arose just beneath the periderm and around the margin of this stromatic area.

In the case of the conidial stroma, large conidial locules were formed within the ectostromatic mat. Conidial locules were also formed within the mats of the entostromatic mycelium, as has been observed in other species of *Eutypella* carried in culture. A number of these conidial locules were formed within and beneath each ectostroma; these later became confluent, giving rise to labyrinthiform chambers. The conidia were identical with those found on agar. A conidial stage similar to this, but with conidia  $20-22 \times 0.75 \mu$ , is described by Saccardo as *Cytosporina cerviculata* Sacc.

The stromata of the perfect stage were widely erumpent, on account of the fact that the hyphae of the ectostroma penetrated and disrupted the periderm over a rather wide area. In the case of the perithecial stromata no conidial cavities were formed within the ectostromatic mat, while within the entostromatic tissue of the bark, perithecial initials, consisting of compact, spherical masses of hyphae, were formed instead of conidial locules. Another interesting observation was that of the occurrence of narrow, cylindrical columns of tissue within the stroma consisting of fine, hyaline, compacted hyphae. Within these areas the bark and periderm cells were entirely absorbed or pushed aside. These canals arose at the same time as, or even before, the perithecial initials. They penetrated both the ectostromatic cap and the entostromatic bark tissue below, and were often seen connected with a perithecial primordium. They were apparently preformed perithecial necks.

#### DIAPORTHE GALERICULATA (TUL.) SACC.

Material of this species was collected on twigs of *Fagus grandifolia* Ehrh. near Ann Arbor. The stromata are minute, thickly scattered, and 0.3-0.5 mm. in diameter. They are erumpent through a split in the periderm in the form of minute, elliptical to fusiform, yellowish-white discs, which contain a few hemispherical, black, roughened ostioles. The perithecia are spherical, 350-400  $\mu$  in diameter, have walls 10-13  $\mu$  thick, and are buried in the bark cortex just beneath the erumpent plug of the disc. There is no blackened zone surrounding the perithecial clusters. The spores (Pl. XIV, fig. 7) are biseriate, cylindrical-ellipsoid, 2-celled, and hyaline. They are slightly constricted at the septum, measure  $21-26 \times 4.5-5.5 \mu$ , and possess a short, hyaline, evanescent appendage,  $5-7 \times 2.5 \mu$ , at each end of the spore.

On December 11, 1923, sprays of ascospores were made from the twigs of *Fagus* mentioned. These twigs had been collected in May (1923) and,

since the spores were not quite mature, were placed in a damp chamber for 4 days previous to making the sprays. Twenty-four hours after being sprayed on the agar all the spores were germinating. The spores were somewhat swollen in their lateral diameter, measuring  $24-26 \times 7.5-8.5 \mu$ , and had thrown out one to two germ tubes,  $3-4 \mu$  in diameter.

On March 3, 1924, a sterile twig of *Fagus* with its base immersed in 6-percent oatmeal agar was inoculated from a single-spore culture. There soon appeared on the surface of the agar local patches of mycelial growth with a whitish to tan color. These patches increased in vertical diameter to produce isolated superficial stromata  $2-4$  mm. in diameter. On May 1 sections of these stromata showed perithecia with mature asci and spores. Subsequent cultures on 6-percent oatmeal agar formed numerous perithecial stromata of the same type. The perfect stage was also produced in abundance on twigs of *Fagus* in culture.

On agar there is very little superficial mycelial growth outside of the pulvinate stromata which are scattered over the surface. These stromata originate as a proliferation of hyphae just within, and on the surface of, the agar, and first appear as pinkish-tan swellings. The aërial hyphae are at first loosely aggregated, but as growth continues they soon become closely compacted. The perithecia arise as small spherical knots of hyphae, both in this central compacted mycelium and in the outer loosely woven hyphae. In the earliest stages seen these knots of hyphae were  $20-25 \mu$  in diameter. Each then consisted of a deeply staining central curved or coiled hypha, surrounded by a layer of ordinary, closely woven, vegetative hyphae. These deeply staining hyphae are very minute ( $1-2 \mu$  in diameter), and their origin and structure were not determined. They are richly filled with protoplasm which takes the stain more readily than that of the vegetative hyphae, and contain many deeply staining nuclei. This deeply staining coil of hyphae increases in size, and the nuclei become more scattered. After the perithecial initials reach a certain size a differentiation takes place. The outer hyphal cells become enlarged and form an outer pseudoparenchymatous tissue of large, thin-walled cells. The central deeply staining hyphae become loosened in the center of the coil and a number of loose ends appear. These hyphae enlarge and appear to give rise to the asci. A thin layer of narrow, deeply staining hyphae remains about the periphery of the perithecial cavity. The formation of the asci and paraphyses within this cavity crushes the outer large-celled layers of hyphae and gives rise to the perithecial wall of flattened pseudoparenchymatous cells. Ascospores from these perithecia on agar germinated normally within 24 hours.

The first evidence of stroma-formation on twigs is the appearance, at various points, of an interwoven mass of hyaline ectostromatic hyphae just beneath the periderm and on the surface of the bark. The bark tissues at this time are rather sparingly penetrated by fine fungous hyphae. After giving rise to this ectostromatic cushion, the hyphae within the bark also

begin to increase in amount beneath it. The growth of the ectostroma produces a compact conical stroma (Pl. XIV, fig. 2) which bursts open the periderm. The perithecial initials arise as spherical knots of hyphae in the developing entostroma beneath. This entostromatic mycelium increases in amount, pushes apart the bark cells, and forms the pustulate immersed stroma of the mature fruit body (Pl. XIV, fig. 1). Along with this development the perithecial necks push upward as outgrowths of the perithecial initials. The outer layers of these columns of hyphae become browned, while the central hyphae become loosened and free ends appear in the central canal. These free hyphal tips are the periphyses of the perithecial neck. These structures push through the ectostroma and become erumpent as the ostioles, which nearly obliterate the erumpent disc of the ectostroma. The ostioles formed in culture were cylindrical and 0.5-1 mm. in length.

No conidial fruiting bodies were found either in agar or in twig cultures of this species. The Tulasnes (17, p. 203) have described an imperfect stage of their *Valsa galericulata* which Saccardo (16, vol. 3, p. 250) calls *Fusicoccum galericulatum* (Tul.) Sacc. It is described as forming small conical, pustulate stromata just beneath the periderm, each containing several conidial locules. The spores are described as fusoid-elliptical,  $6-10 \times 3.5-4 \mu$ . This fungus was called *Myxofusicoccum galericulatum* (Tul.) Died. by Diedicke (2, p. 318) in 1912. It was placed in the new genus *Malacostroma* by von Höhnelt (10, p. 355) in 1917. Von Höhnelt gives as a synonym *Myxosporium carneum* Thüm., which has spores described as measuring  $15-17 \times 3.5-4.5 \mu$ . The writer has found pycnidia associated with *Diaporthe galericulata* in nature, with a structure very similar to those of the form referred to. The tissue of the stroma was more or less blackened and contained one or more locules with blackened walls. The conidia formed in these locules were, however, long-cylindrical, hyaline, 1-celled, hamate or bent at the tip, and measured  $13-21 \times 1-1.5 \mu$ . The occurrence of such spores suggests that the imperfect stage may be a *Phomopsis*, and these the Beta type of spore, which has not previously been noted.

#### DIAPORTHE OBSCURA (Pk.) SACC.

Material of this species was collected on *Rubus* sp. near Ann Arbor. The stromata are rather widely scattered; are composed almost entirely of fungous tissue; are seated just beneath the periderm; and are erumpent through an elongated slit in the periderm. The cluster of short, cylindrical, punctate ostioles is barely erumpent. The perithecia are nearly spherical,  $180-200 \times 130-180 \mu$ , with long necks and coriaceous walls. They are buried in a stroma of grayish hyphae seated within the bark tissues. The asci are clavate, and measure  $23-28 \times 5-7 \mu$ . The spores (Pl. XIV, fig. 8) are biserial, clavate to pyriform in shape, hyaline, and with an obscure septum near the narrowed end. The spores measure  $6.5-7.5 \times 2-2.5 \mu$ .

Sprays of ascospores from these *Rubus* twigs were made on December

20, 1923. Twenty-four hours later the spores were germinating. The spores were swollen in an irregular fashion, thus presenting various odd shapes. The swollen spores measured about  $13 \times 10 \mu$ , and each formed two to four germ tubes  $2.5 \mu$  in diameter.

A culture on 6-percent oatmeal agar was made in this case from a cluster of asci. The growth was at first apparent merely as a browning of the surface of the agar. Small, grayish superficial stromata, some 0.5 mm. in diameter, soon appeared and developed into elongated-cylindrical to irregularly shaped, stromatic formations, 1–3 mm. in height, and 0.5–1 mm. in diameter. These stromatic bodies (Pl. XIV, fig. 3) were composed of interwoven brown-walled hyphae which formed a plechtenchymatic tissue. Isolated areas of tissue within this stroma remained hyaline and thin-walled and capable of further development. In these spherical to irregular-shaped areas were formed the conidial locules. The central portions of these areas of hyaline tissue seemed first to disintegrate, leaving a mass of large refractive granules. The conidia were then formed in large numbers from the lateral hyphae, and soon completely filled the cavity. The hyphae about the cavity became compacted; their walls became more heavily thickened and browned and took on the appearance of a pseudoparenchymatous, wall-like tissue. The conidia (Pl. XIV, fig. 4) were 1-celled, hyaline, narrow-fusiform to elliptical, and measured  $4-7 \times 1-2 \mu$ . The conidia show a wide range of variation in shape from elliptical to fusiform-cylindric, and, although all intermediate forms exist, it can be seen that an elimination of such transitional forms with a production of only the extreme types would result in a Phomopsis type of spore-production.

On January 9, 1924, stems of *Rubus* sp. with their bases immersed in 6-percent oatmeal agar were inoculated from the culture on agar. Pustules were formed within three to four weeks on both the agar and the stems. On May 14, perithecia with mature asci and spores were found on the stems, and in a few cases on the agar near the stems. The groups of perithecia on the agar were superficial and were more or less surrounded by a stromatic tissue.

Superficially the pycnidial and perithecial stromata seem to arise in the same manner, but an examination of the young stages shows their origin to be different. The pycnidia arise from a knot of mycelium within the cortex. This mycelial mass (Pl. XIV, fig. 5) increases in size and forms a spherical, conical, or flask-shaped stroma. The growth of this stroma finally bursts open the periderm. Its structure is similar to that of the stromatic formations on agar. The outer cell layers have heavy brown walls and form a pseudoparenchymatous outer layer. A cavity is formed within this stroma, usually toward the base, by the loosening of the hyphae, and numerous spores are abstracted similar to those formed on agar (Pl. XIV, fig. 6).

In the case of the perithecial stroma, the perithecial initials arise before, or at the same time as, the beginnings of the entostromatic mycelium



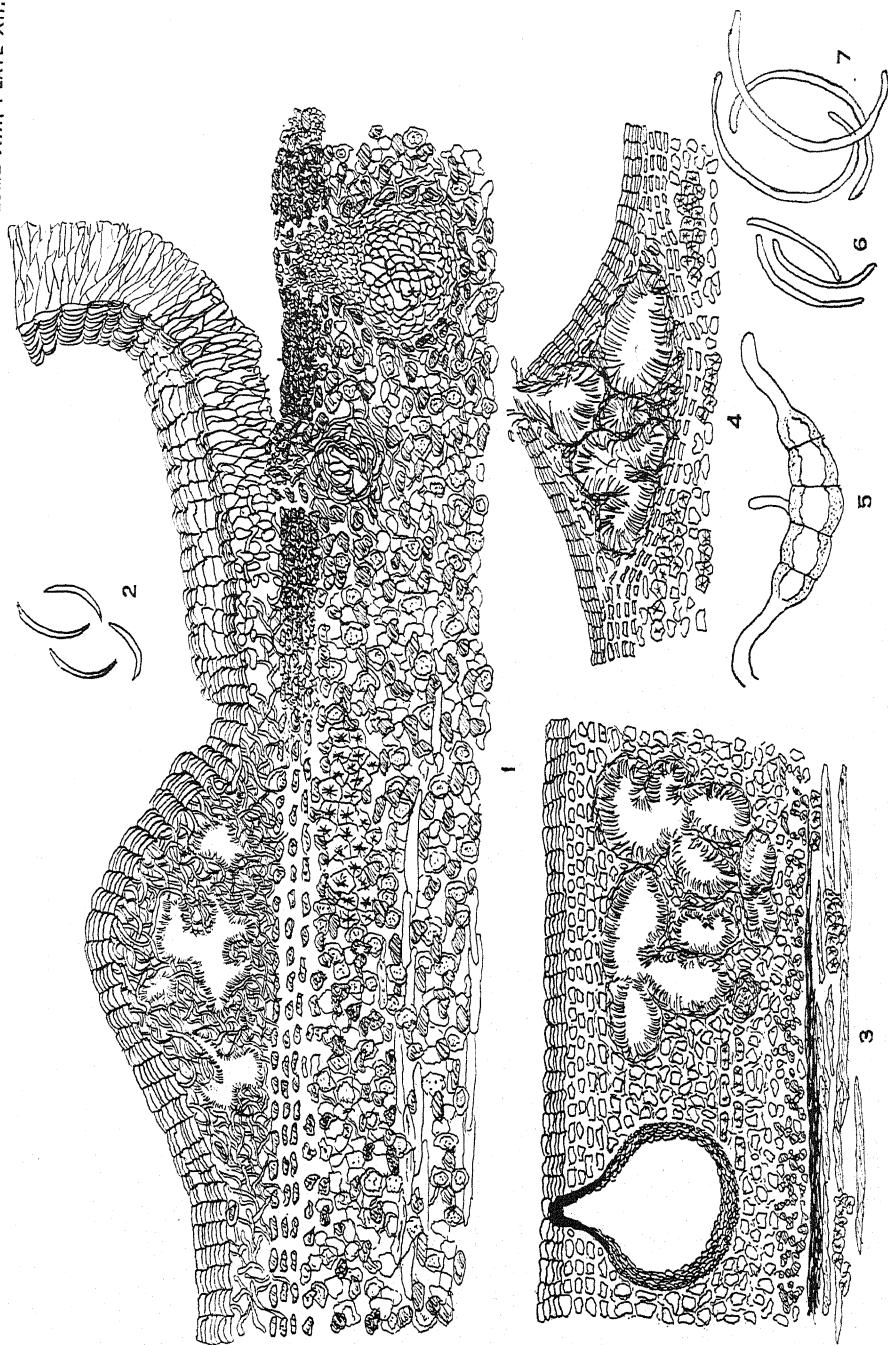
within the bark cortex. As the perithecia develop, this mycelium increases between and around them, spreading apart and absorbing the bark cells. Eventually a well developed stroma is formed (Pl. XIV, fig. 5) composed entirely of fungous tissue, with the perithecia immersed in its base. At maturity the perithecial walls are composed of several layers of black, heavy-walled pseudoparenchyma. The perithecial necks, which penetrate the stroma above, are long and narrow and have an outer wall of blackened pseudoparenchyma. The ostioles formed in culture were 0.5-1 mm. in length and filiform.

On account of its well developed stroma and its unequally two-celled spores, this species would fall in the genus *Apioportha* of von Höhnelt (Sitzungsb. Akad. Wiss. Wien Math.-naturw. Kl. 126: 381).

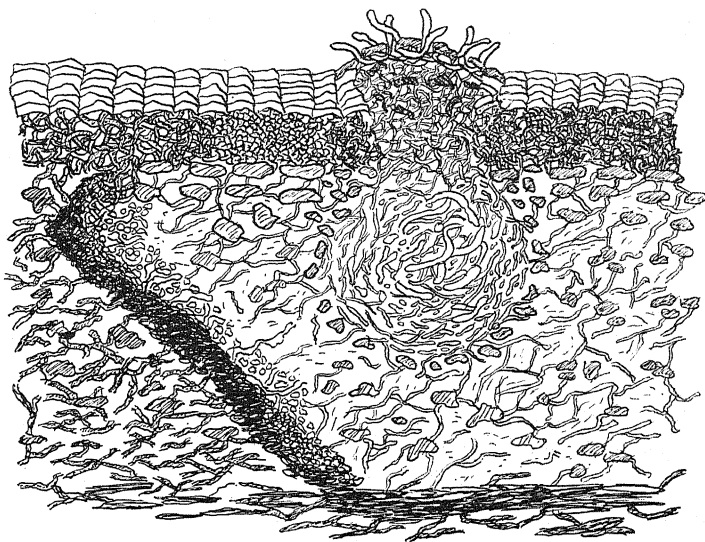
In conclusion, the writer wishes to express his appreciation to Dr. C. H. Kauffman for his helpful suggestions throughout this work.

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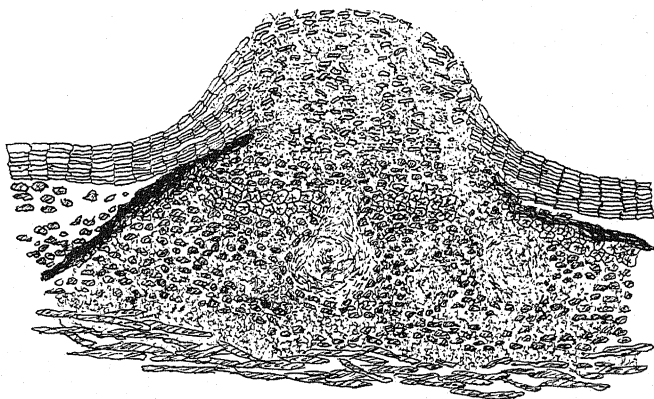
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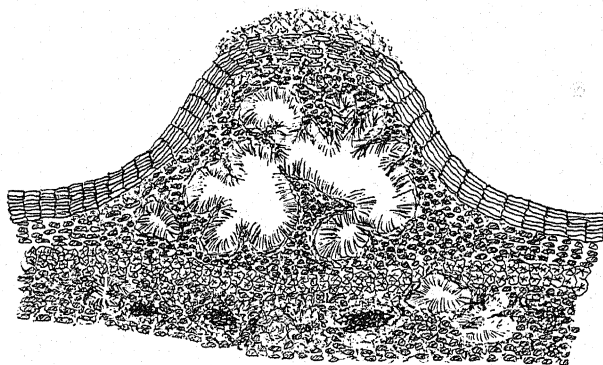




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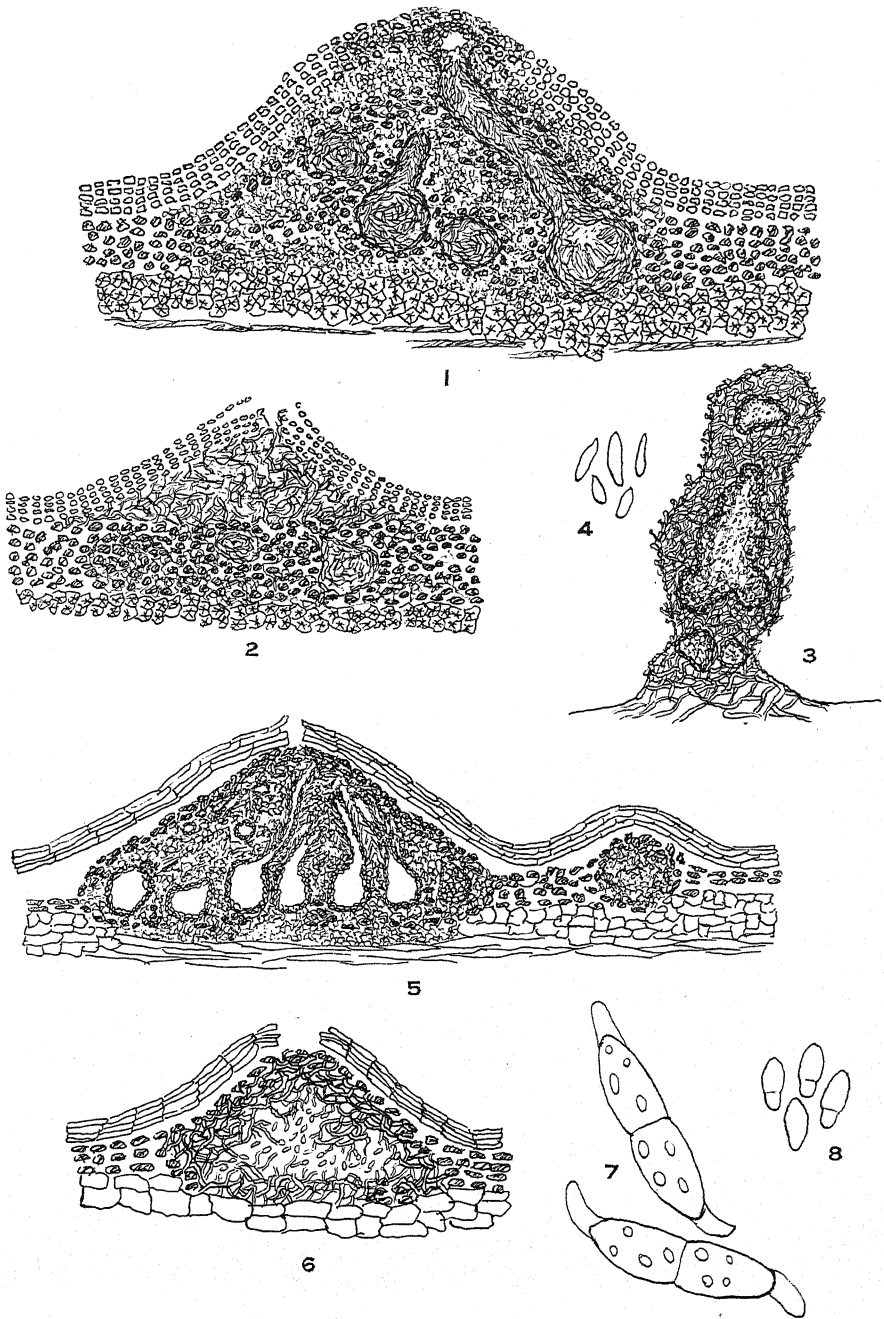


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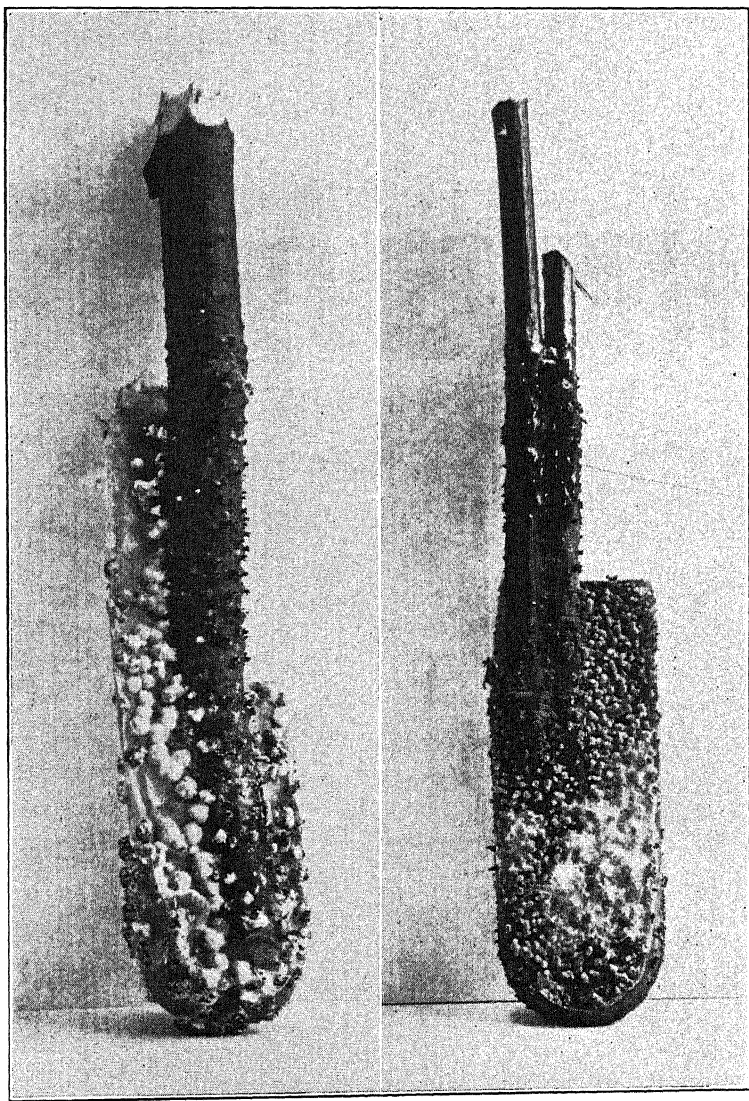
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WEHMEYER: STROMATIC SPHAERIALES





WEHMEYER: STROMATIC SPHAERIALES





## EXPLANATION OF PLATES

## PLATE XII

FIG. 1. Vertical section of the margin of a perithecial stroma of *Diatrype stigma* (Hoff.) de Not., showing various stages in its development.

FIG. 2. Conidia of *D. stigma*.

FIG. 3. Vertical section of perithecial stroma of *Cryptosphaeria eunomia* (Fr.) Grev., showing entostromatic conidial locules.

FIG. 4. Ectostromatic conidial locules of *C. eunomia* formed on sterile twigs in culture.

FIG. 5. Germinating ascospore of *C. eunomia*.

FIG. 6. Short type of conidia formed on sterile twigs by *C. eunomia*.

FIG. 7. Normal-type of conidia of *C. eunomia*.

## PLATE XIII

FIG. 1. Vertical section of portion of perithecial stroma of *Cryptosphaeria populina* (Pers.) Sacc.

FIG. 2. Vertical section of young perithecial stroma of *Eutypella cerviculata* (Fr.) Sacc., showing perithecial initials and preformed perithecial necks.

FIG. 3. Conidial fruiting body of *E. cerviculata*, showing both ectostromatic and entostromatic locules.

FIG. 4. Conidia of *E. cerviculata*.

## PLATE XIV

FIG. 1. Vertical section of young perithecial stroma of *Diaporthe galericulata* (Tul.) Sacc.

FIG. 2. Earlier stage of perithecial stroma of *D. galericulata* showing ectostromatic tissue.

FIG. 3. Vertical section of the type of conidial fruiting body formed by *D. obscura* (Pk.) Sacc. on oatmeal agar.

FIG. 4. Conidia of *D. obscura*.

FIG. 5. Vertical section showing perithecial stroma and pycnidial primordium of *D. obscura* on sterile stem of *Rubus* sp.

FIG. 6. Vertical section of pycnidium of *D. obscura* on sterile stem of *Rubus* sp.

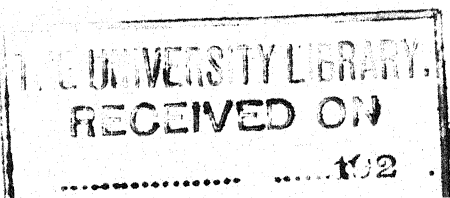
FIG. 7. Ascospores of *D. galericulata*.

FIG. 8. Ascospores of *D. obscura*.

## PLATE XV

Left: Culture of *Diaporthe galericulata* on twig of *Fagus grandifolia*. Perithecial stromata on agar and erumpent fascicles of ostioles on the twig.

Right: Culture of *Diaporthe obscura* on stem of *Rubus* sp. with base immersed in 6-percent oatmeal agar. Pycnidial stromata on agar, pustules of perithecial stromata on the stem.



# CYTOLOGICAL STUDIES ON *PHASEOLUS VULGARIS*

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## INTRODUCTION

The earlier researches on the embryology of the Leguminosae begin with the work of Schleiden and Vogel (18). They described floral development and endosperm-formation in more than 50 species of this group. Following them was Tulasne (23), who described in a short paragraph the embryo of *Lathyrus Aphaca*, and noted that the basal cells of the filamentous suspensor are swollen. Hofmeister (8) briefly described embryo-formation in a number of Leguminosae, and observed peculiar outpocketings in the pollen tube of *Lupinus*.

Hegelmaier (7) reported on the embryo sac and embryo in several species of *Lupinus*. He found that the anterior end of the embryo sac contains only the synergids, posterior to them being the "Kern des Keimsackes." The "egg apparatus," a multinucleate structure which presumably gives rise to the embryo, he located at the median portion of the embryo sac, at the point of its greatest curvature. He was uncertain about the antipodals, and found multinucleate cells in the filamentous suspensor. Hegelmaier's work was repeated in the same year by Strasburger (20), who found no deviation from the typical structure in the embryo sac of *Lupinus*. Hegelmaier evidently confused endosperm nuclei with the egg apparatus. In the following year Guignard (6) published his investigations of the embryo sac and embryo in about 40 species of Leguminosae. In *Phaseolus multiflorus* he found a single hypodermal cell which divides into an "apical" and a "sub-apical" cell. The apical cell later forms a layer of tissue a few cells in thickness; the sub-apical or sporogenous cell gives rise to an axial row of three macrospores. The innermost of these macrospores forms a typical 7-celled embryo sac with ephemeral antipodals. The egg by two transverse divisions forms a 3-celled pro-embryo, the terminal cell of which develops the embryo, the other two developing the filamentous suspensor.

Saxton (17) studied the development of the embryo sac and embryo of *Cassia tomentosa*. He found a deeply imbedded macrospore mother cell which gives rise to a linear tetrad of macrospores. The third macrospore from the micropylar end develops into a 7-celled macrogametophyte, the antipodals lying in a row in a tubular prolongation of the embryo sac and seemingly functioning as a conducting tissue. Martin (12) investigated five species of *Trifolium*, *Medicago*, and *Vicia*. In all these he found a multicellular archesporium, the production of a linear tetrad of macrospores,

and ephemeral antipodals. Lastly, five varieties of *Phaseolus vulgaris* were reported on by Miss Brown (1). She found a single hypodermal cell which divides into an axial row of three macrospores, the innermost one forming a typical 8-nucleate embryo sac. She noted that the antipodals in these varieties disappear at about the time of fertilization, the synergids disintegrating soon after. Her account of embryo-development in *Phaseolus vulgaris* corresponds to that of Guignard for *Phaseolus multiflorus*.

#### MATERIAL AND METHODS

Buds, pistils, and young fruits and seeds were obtained from the following varieties of *Phaseolus vulgaris* L.: "Yellow Eye", "Black Wax", "Kentucky Wonder", and "Stringless Green Pod." The seeds of two types of the Yellow Eye variety were from pedigreed stock kindly furnished by Dr. K. Sax, of the Maine Agricultural Experiment Station; the others were commercial varieties from the L. L. Olds Seed Company, Madison, Wisconsin. The plants were grown in the botanical greenhouses of the University of Wisconsin, and a number of plantings were made from October, 1923, through January, 1925.

The material, at different stages of development, was collected at short intervals during the day and often at night, and each stage was fixed separately. As fixatives, Flemming's medium and strong solutions, Licent's chrom-acetic-formalin solution, Bouin's fixative, 1-percent chrom-acetic acid, and Taylor's modification of Flemming's solution were used. The youngest buds were placed intact in the fixing fluid; older buds were dissected, and the pistils were fixed separately. In the cases of maturer fruits, the seeds were picked out and placed in the fixing fluid by themselves to insure penetration. Licent's solution (80 parts 2-percent chromic acid, 5 parts glacial acetic acid, 15 parts commercial formalin) gave the best fixation of material in all stages of development.

Sections from 6 to 10 microns in thickness were cut. These were stained with Heidenhain's iron-alum haematoxylin, or with Flemming's triple stain.

With the exception of slight variations in the longevity of the synergids, no significant differences were observed in the stages of the life history studied as between the above-mentioned varieties. The description which follows is based chiefly on material from Yellow Eye, but applies also to the other varieties studied.

#### OBSERVATIONS

##### Microgametophyte

The archesporium of *Phaseolus vulgaris* becomes differentiated in the anther at a very early period. When it can be first definitely distinguished it consists of a hypodermal column, consisting in cross section of from three to five cells. The outermost cells of this column cut off the primary

parietal cells, which in turn divide to form a layer about three cells in thickness (Pl. XVI, fig. 1). The innermost layer of these cells later forms the tapetum. No divisions were seen in these cells, the conclusions as to their history being drawn from the shape and size of the cells in question. The tapetum is not differentiated until the heterotypic prophase are well advanced.

The pollen mother cells undergo a period of growth which lasts for some time after the divisions of the primary parietal cells (fig. 2). Their nuclei become large and show traces of a very open and delicate network. The chromatic material soon takes on the form of a loose reticulum, in the meshes of which heavily staining, spherical bodies appear (fig. 4). As the nucleus grows, the material of this reticulum aggregates to form a beaded thread whose double nature is quite distinct (fig. 5). The thread then begins to thicken, and finally contracts to form a very tight knot (figs. 6-8). At this stage the pollen mother cells begin to separate from each other, but still retain their angular shape. When the cells become entirely rounded and are separated from each other by what appears to be a gelatinous matrix (fig. 3), the spireme becomes a loosely spread-out double strand (fig. 9). In the next stages observed, the spireme thickens, the two strands seemingly fusing into one. The spireme is then broken into short lengths the number of which could not be ascertained with any degree of certitude (figs. 10, 11). The short lengths of the spireme now contract and thicken, and arrange themselves peripherally in the nucleus. In advanced diakinesis they assume definite shapes, transitional to those of the chromosomes in the equatorial-plate and metaphase stages (figs. 12, 13).

The spindle during the equatorial-plate and later stages is bipolar, and the chromosomes form a fairly regular equatorial plate. In the telophases the chromosomes anastomose, forming a very dense reticulum (fig. 14). The daughter nuclei then pass into what seems to be a resting condition, although interkinesis lasts but a short time. Details of the homoeotypic division were not followed, but the two spindles in a pollen mother cell are at right angles to each other. The four daughter nuclei of the second division pass into the resting state.

The mother cell wall becomes greatly thickened soon after the four newly formed nuclei reach the resting stage. Farr (3) in his earlier work on the cytokinesis of pollen mother cells thought that this thickening results from a gelatinization of the cell wall just prior to the division of the cell by furrows. Gates (5) in his recent work on pollen-tetrad wall-formation in *Lathraea*, describes the thickening as due to a "special wall" secreted by the cytoplasm, which seems to be independent of the original mother cell wall although laid down in contact with the latter. He figures the "special wall" as detached wholly or in part from the original mother-cell wall, thus remaining in contact with the cytoplasm. In the material from which the present study was made, not a single case of such pulling away was observed,

and there appears to be no structural difference between the outer and the inner parts of the thick mother-cell wall. In fact, the walls are so homogeneous that it is often difficult to find any line of demarcation between the thick walls of adjoining cells.

Division of the tetranucleate cell begins by a pinching-in of the cytoplasm at the periphery midway between each two nuclei, followed by a gradual diminution in density of the cytoplasm along the path of cleavage. Gates (5) finds that the furrowing begins, not by an active invagination of the cytoplasm, but rather by a passive deposition of wall material in these positions, the latter stages of this deposition being supplemented by active furrowing on the part of the cytoplasm. He also finds in these furrows delicate cell walls which can be demonstrated only by special wall stains.

Separating walls in tetrad-formation in *Phaseolus vulgaris* are easily seen in the more advanced stages of development (Pl. XVII, fig. 18). These walls thicken rapidly and appear to be of the same material as the surrounding thick mother-cell wall, so that the microspores in a fully formed tetrad appear to be set in a solid gelatinous matrix (fig. 19).

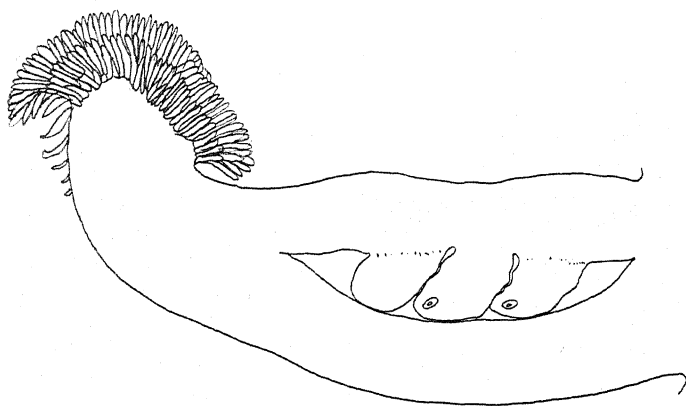
Both the surrounding and the separating walls disintegrate soon after the formation of the microspores, which then lie loose in the anther sac. This disintegration goes on very rapidly, so that in the same anther one sac may show pollen tetrads, each within its matrix, while in the adjoining sac all the microspores are separate without any trace of common walls.

The mature pollen grain is triangular in shape, and has a very thick wall with a germ pore at each rounded angle (fig. 21). The cytoplasm is exceedingly vacuolate, and a large tube nucleus is in the center of the grain. To one side is the smaller pyramidal generative cell, definitely marked off by a membrane from the cytoplasm of the tube cell. Pollen of the Yellow Eye variety shows a marked tendency to germinate within the anther (at least under greenhouse conditions). Many pollen grains begin to germinate four to five hours before pollination normally occurs, and some develop tubes of considerable length within the anther. Figures 15 and 16 (Pl. XVI) were drawn from material from an anther which was crushed on a slide in 1-percent acetic acid to which a small drop of gentian violet was added. Whether such germinated pollen plays any part in fertilization was not determined.

#### Ovule and Macrogametophyte

From 3 to 5 ovules—as many as 10 in Kentucky Wonder—are borne alternately on the two margins of the placenta, which is located on the inner dorsal surface of the laterally flattened ovary. The ovules first appear as slight protuberances under the inner cell layer of the ovary, the raised portion of the latter becoming the epidermal layer of the nucellus. As the ovules develop, they fill the ovarian cavity and begin to incline toward the stylar end (text fig. 1). At this stage two lateral swellings—the primordia of the integuments—appear on each side of the ovule (Pl. XVII, fig. 23).

The outer integument, particularly on the side away from the placenta, grows much faster than the inner one, and by the time the ovule is turned at right angles to the funiculus the outer integument half envelops the nucellus (fig. 24). Further turning of the young ovule, which is due to the more rapid division and growth of cells on one side than on the other, takes place slowly, but the outer integument maintains its rate of development



TEXT FIG. 1. Very young pistil with ovules.  $\times 35$ .

and grows around the nucellus in such a manner that the micropyle reaches a position parallel to the funiculus while the apex of the nucellus is still nearly at right angles to the latter (fig. 25). As development goes on, the inner integument, on the funicular side, also reaches the apex of the nucellus, but on the opposite side its development stops early so that it reaches only to the level of the egg in the mature macrogametophyte (Pl. XVIII, fig. 36). The outer integument at maturity is a massive structure; the inner is only two cells in thickness, except near its upper margin where it is four or five cells thick.

The macrospore mother cell is differentiated very early in the development of the ovule, even before the primordia of the integuments appear. When it can first be definitely distinguished it is a somewhat larger cell than its neighbors, located in the axial row of the nucellus (Pl. XVII, fig. 26). Occasionally two or more similar large cells may be seen grouped together, but in no case does more than one continue to develop. The young macrospore mother cell is separated from the epidermis by a single layer of cells, the origin of which could not be ascertained. Guignard (6) found that the archesporium in *Lupinus*, *Phaseolus multiflorus*, and other forms, originates as a hypodermal cell which divides transversely, the inner cell becoming the macrospore mother cell. Miss Brown (1) was unable to decide whether the hypodermal archesporial cell in *P. vulgaris* functions directly as the macrospore mother cell or divides first into a primary parietal cell and a sporogenous cell. No division figures were observed in this study that would

indicate the origin of the hypodermal layer, but the position of the cells directly above the macrospore mother cell is very similar to the position of those pictured by Guignard after the primary parietal cell has undergone vertical division.

The macrospore mother cell grows very rapidly and becomes conspicuous for its size and its densely staining cytoplasm. The nucleus is large and is located near the micropylar end of the cell (fig. 27). The outer integument has extended around the apex of the nucellus by the time the macrospore mother cell is fully grown, and the micropyle is now in the position in which it will be found when the ovule is mature. The macrospore mother cell now divides transversely. As a rule the outer of the two daughter cells does not divide, but occasionally an aborted transverse spindle may be seen in it (fig. 29). The inner of the two daughter cells formed from the macrospore mother cell grows and in its turn divides. Thus a linear row of three cells is typically formed (fig. 30). Nuclear division occurs at times in the second cell of this triad (fig. 31)—that is, in one of the macrospores formed by the second division. Guignard found that cell division sometimes follows such a nuclear division in *Phaseolus multiflorus*.

The chalazal macrospore functions and develops into the embryo sac in all four varieties studied. Miss Brown (1) found the same thing to be true in the five varieties of *P. vulgaris* studied by her. Guignard (6) observed the formation of only three macrospores, the chalazal one functioning, in *P. multiflorus* and in *Medicago arborea*. In a number of other Leguminosae, however, he found four macrospores formed and noted variations in different genera as to which of the four macrospores is functional. Thus, in several species of *Acacia* it is the third one from the micropylar end that functions; in *Cercis* and *Orobis*, the chalazal macrospore of the tetrad forms the embryo sac. Strasburger (20) reported the latter condition for *Lupinus* and *Orobis*, and Martin (12) for *Medicago sativa*, *Vicia americana*, and three species of *Trifolium*. The third macrospore functions, according to Saxton (17), in *Cassia tomentosa*.

The functional macrospore in *Phaseolus vulgaris* grows considerably in length before its nucleus divides (fig. 30). The other two macrospores degenerate rapidly, and but a trace of them is left by the time the nucleus of the functional spore has divided. In a number of cases, however, the degeneration of the non-functional macrospores is not so rapid, and they can still be clearly seen even at the binucleate stage of the embryo sac (fig. 32).

The eight nuclei of the embryo sac are formed by three successive divisions in the usual manner. The two polar nuclei migrate from their original positions and meet in the upper third of the young embryo sac, which now shows a distinct curvature. Cell-formation takes place at both the antipodal and the micropylar ends of the embryo sac at this stage, and a large vacuole is formed between the polar nuclei and the antipodal cells.



This vacuole grows in size and seems to push the polar nuclei upward, until they finally come to lie in close proximity to the egg apparatus (Pl. XVIII, figs. 35, 36). The antipodals soon degenerate, and hardly a trace of them can be found in the mature embryo sac, which becomes sharply curved, the curvature being almost at right angles to the long axis of the ovule (fig. 36). The synergids also disappear before fertilization in the Yellow Eye variety, but show a tendency to persist until after fertilization in the other varieties studied.

The apical portion of the nucellus is gradually absorbed during the development of the embryo sac, and at maturity the sides of the latter—from the point of its greatest curvature to the level of the egg apparatus—are in direct contact with the inner integument. The epidermal layer of the nucellus, which was originally a part of the lining of the ovarian cavity, persists as a hood over the apex of the embryo sac (fig. 36) until the embryo reaches a fairly advanced stage.

The lower half of the embryo sac forms a tubular prolongation into the larger remaining portion of the nucellus, whose cells become much elongated and are slowly crushed by the encroaching embryo sac. However, they are not absorbed immediately, but persist until the cotyledons of the embryo have developed to a considerable extent.

This study has shown that the closest similarity exists in the development of the embryo sac between the four varieties of *P. vulgaris* discussed in this paper and the varieties studied by Miss Brown. The development of the macrogametophyte is preceded, in all these varieties, by the formation of an axial row of three macrospores, instead of the usual four, and this feature has also been found in *P. multiflorus* (Guignard, 6), the only other species of *Phaseolus* studied to date. A close relationship is shown between *P. vulgaris* and other Papilionoideae in the ephemeral antipodals, in the early disintegration of the synergids, and in the swollen or otherwise modified basal cells of the suspensor. These features have been observed by numerous investigators already cited, in *Lupinus*, *Lathyrus*, *Trifolium*, *Medicago*, *Vicia*, and other genera.

### Fertilization

The embryo sac at the time of fertilization usually consists of but two cells—the egg and the primary endosperm cell. The egg is large, pear-shaped, and sharply pointed at its micropylar end. Its nucleus, in the resting condition, is located near the broad chalazal end, and a large vacuole fills the entire space between the nucleus and the pointed apex of the cell. In case the synergids still persist, they are both located at one side of the egg. The polar nuclei are a short distance below. In some embryo sacs they are still a short distance apart; in other sacs they are beginning to fuse. Most commonly these nuclei are in close contact with each other but have not yet begun to fuse.

Comparatively few pollen tubes reach the ovary. The great majority of the tubes do not even penetrate the stigma, but grow along its outer surface. The few that penetrate the hollow style grow down its cavity and then along the surface of the glandular placenta. But a single pollen tube enters each micropyle, and the supernumerary ones grow onward to the basal end of the ovarian cavity where their distal portions eventually disintegrate. Both male nuclei, and occasionally the tube nucleus, may be seen in these tubes for some time after they reach the end of the ovary (Pl. XVII, fig. 22).

The pollen tube enters the embryo sac directly from the micropyle, boring its way through the remaining epidermal layer of the nucellus, and its end lies at one side of, and in close proximity to, the egg. When the synergids are present the pollen tube enters between one of them and the egg, without destroying the synergids. The tip of the tube, usually slender as compared with the rest of its length, reaches the apex of the egg and often overlaps it (Pl. XVIII, fig. 41). Figure 37 represents such a pollen tube with the two male nuclei not yet discharged and the tube nucleus, which was cut by the knife, faintly showing. The rest of the tube nucleus is in an adjoining section. The two polar nuclei are already partially fused.

A pollen tube, entering the micropyle, penetrates the embryo sac within 8 or 9 hours after pollination, and fertilization takes place soon after. No free male nuclei were observed in the cytoplasm, but from the position of the tip of the pollen tube it appears that they are discharged into the space between the egg and the polar nuclei, whence one migrates toward the egg and the other to the neighborhood of the polar nuclei. The tube nucleus usually remains inside the tube, but at times it is apparently discharged together with the male nuclei. Figure 40 shows a pollen tube without the tube nucleus, while two small nuclei are apparently fusing with one of the polar nuclei in the same embryo sac. Since a third nucleus is fusing with the egg, one of the three may be the tube nucleus, which was probably carried down with the two male nuclei when the latter were discharged.

The male nuclei are very small in comparison with the egg and polar nuclei, and retain their spherical shape during fusion. In the primary endosperm cell, the male nucleus fuses with one of the polar nuclei, which latter unites simultaneously with the other polar nucleus, except in cases in which the fusion of the polar nuclei began before fertilization. Fusion of the egg nucleus with the second male nucleus goes on at the same time, and all nuclei are in the resting stage at the time of union (figs. 38-40).

The primary endosperm nucleus (formed by the triple fusion) undergoes division immediately after fertilization; the zygote nucleus divides much later (fig. 42). The prophases of division are difficult to follow in the egg, because of the comparatively small size of the nucleus and of the limited

amount of chromatic material that appears. In the primary endosperm nucleus, however, these stages may be easily observed. There is no definite indication that more than a single spireme is formed in this nucleus, and it is even difficult at this time to determine the original location of the male nucleus. Moreover, there seems to be a tendency for most of the chromatic material to aggregate inside the still faintly visible limits of one polar nucleus, instead of being evenly spread out through the cavities of both (Pl. XVIII, figs. 43, 44; Pl. XIX, fig. 45). The nucleoli of the polar nuclei may be either fused (Pl. XVIII, fig. 44) or separate (fig. 43) at this time.

In other groups of the Leguminosae variations have been noted in the time relations between the division of the zygote and that of the primary endosperm nucleus. Strasburger (20) found the zygote and endosperm nuclei dividing simultaneously in *Lupinus*, and Guignard (6) observed the same thing in *Phaseolus multiflorus*. Martin (12) noted that the division of the endosperm nucleus in *Trifolium* usually precedes that of the zygote nucleus, although it occasionally follows. In *Vicia americana* he found the two nuclei dividing at the same time.

In the history of fertilization and of related processes, *Phaseolus vulgaris* agrees with the majority of the dicotyledons. The particular features are the rapidity with which fertilization occurs, and the regularity in time intervals between successive stages of development, from pollination to maturity of the embryo. Under greenhouse conditions pollination occurs at about the time the flower opens, which takes place usually between 1:30 and 2:30 A.M. Fertilization is accomplished in about 8 or 9 hours, and is followed immediately by the division of the primary endosperm nucleus, the division of the zygote nucleus occurring later. Fifteen or sixteen hours after the opening of the flower, 3- or 4-celled embryos are to be observed. The rapidity of these processes may be dependent, to a considerable extent, upon the relatively high greenhouse temperatures. Martin (12) has already observed the effect of temperature on the time relations between pollination and fertilization in *Trifolium pratense*. He found that flowers pollinated during the high temperature of July often showed 3-celled embryos about 18 hours after pollination. In October, on the other hand, from 35 to 50 hours was required for the attainment of this stage. The lengthening of the period is due largely to a delay in tube-development, since during the cooler weather pollen placed on the stigma at 3:00 P.M. was found dormant at 9:00 A.M. the following day.

### Embryo

The division of the zygote is transverse (Pl. XIX, figs. 47-49). The succeeding division is also transverse, thus resulting in the formation of a filament of three cells (fig. 50). Miss Brown (1) found that the second division takes place in the basal cell. The third division is vertical, occurring in the terminal cell (fig. 51), and this is followed by vertical divisions in the other two cells of the filament (fig. 52). The order of the succeeding

divisions was not determined, but Miss Brown found that the next division may occur in a longitudinal plane perpendicular to that of the divisions last mentioned, or that it may be preceded by several transverse divisions; in either case, an embryo is formed of seven or eight tiers, each of four cells.

From now on, division is active only in the apical tier of cells. A series of anticlinal divisions in this layer is followed by periclinal divisions, which latter cut off the dermatogen (fig. 53). In *Phaseolus multiflorus* Guignard (6) found that the dermatogen is cut off later than the octant stage, and Martin (12) found the same to be true in *Trifolium*, *Medicago*, and *Vicia*.

The suspensor develops from the basal and median cells of the 3-celled pro-embryo, the terminal cell alone giving rise to the embryo proper. Multiplication of the suspensor cells ceases at about the time that the dermatogen begins to differentiate; the cells of the two basal tiers become elongated and later swell, assuming a globoid shape and persisting until very late in the history of the embryo. The swollen basal cell of the suspensor becomes multinucleate, the nuclei being near together (fig. 55). There is no distinct demarcation between the embryo and the filamentous suspensor until the hypocotyl reaches an advanced state of differentiation.

Swollen or modified suspensor cells have been found by many investigators in various Leguminosae. Tulasne (23) described them in *Lathyrus Aphaca*; Hofmeister (8) in *Astragalus*; Hegelmaier (7) and Strasburger (20) in *Lupinus*, and Guignard (6) in *Orobus* and *Pisum*. Martin (12) observed multinucleate suspensor cells in *Vicia americana*, and Hegelmaier, Strasburger, and Guignard note similar observations in the genera studied by them.

After the cutting off of the dermatogen, multiplication of cells continues in the apical region until a massive pear-shaped embryo is formed. The cotyledons begin to appear eleven or twelve days after pollination, when the young seed is about 4 mm. in length. They grow rapidly, digesting the endosperm, and by about fifteen days after pollination they almost fill the entire space inside the integuments. The seed is then about 6 mm. long and nearly 4 mm. wide. The other parts of the embryo are also differentiated early, and at this time the hypocotyl, the epicotyl, and the primordia of the first two secondary leaves are clearly visible. The suspensor is still conspicuous, but the endosperm has by this time been almost entirely digested, and the little that remains of it is to be found near the chalazal end of the seed cavity.

### Endosperm

The primary endosperm nucleus divides before the zygote nucleus in all cases observed (Pl. XVIII, fig. 42), and Miss Brown (1) found the same to be true in her study of *Phaseolus vulgaris*. The time intervening between the first division of the primary endosperm nucleus and that of the zygote nucleus varies considerably. Thus, with the zygote nucleus in metaphase, 4 nuclei were found in the endosperm in one case; in another case, 8 were

counted; in still another, with the zygote nucleus in telophase, the endosperm showed 7 free nuclei; and in one preparation 6 nuclei were present in the endosperm when the first nuclear division in the zygote was completed. This irregularity in the number of endosperm nuclei also indicates that successive divisions in the endosperm are not simultaneous. Further evidence of this fact is to be found in the 40- or 50-nucleate stage, when nuclei in all stages of division as well as in the resting condition may be found in the same preparation.

The endosperm forms a peripheral sac which eventually extends to the chalaza as growth progresses. Cell division in the endosperm occurs only in that portion of it which is directly in contact with the embryo. It begins about the time the dermatogen is differentiated, and gradually progresses toward the chalaza as the embryo increases in size. The cell arrangement in this part of the endosperm gives the appearance of a very loose structure. All traces of the endosperm have disappeared when the embryo reaches maturity.

### Chromosome Number

Little work has been done to the present time on chromosomes and chromosome numbers in the Leguminosae. Most of the previous investigators have concerned themselves with the genus *Vicia*, a few species of which have been thoroughly studied. Němec (13, 14), Lundegårdh (10, 11), and Sharp (19) reported 12 as the diploid number of chromosomes in *Vicia Faba*. Fraser and Snell (4) reported 14 chromosomes in the same species; but Sakamura (15, 16) decided that the diploid number is 12. The last-named author found that one pair of chromosomes is much larger than the rest, and is marked by subterminal as well as median constrictions. He suggested that Fraser and Snell's erroneous count was due to these constrictions. He also found 12 chromosomes in *Vicia cracca*, *V. pseudorobus*, and *V. sativa*; 14 in *V. atropurpurea* and *V. pseudocracca*, and 24 in *V. unijuga*. In *Lathyrus*, *Lens esculenta*, and *Pisum sativum* the diploid number, according to Sakamura, is also 14. 14 chromosomes were found in *Pisum* also by Cannon (2), Němec (13), Strasburger (21), and Kemp (9). Saxton (17) found 12 chromosomes in *Cassia tomentosa*. No published account of the chromosome number in *Phaseolus vulgaris* has been found.

The diploid number of chromosomes in *P. vulgaris*, as determined in the present study, is 22. Twenty-one preparations of root tips, from twenty seedlings of four varieties, were examined, and the chromosomes of about sixty equatorial plates were counted. In each equatorial plate 22 chromosomes were found (Pl. XIX, figs. 56-59). A few chromosomes are rod-shaped, but the majority are more or less curved, four chromosomes usually being more curved than the rest. In division figures in developing petals, the chromosomes (fig. 60) are shorter and thicker than in the cells of root tips.

Three preparations showing heterotypic divisions were obtained from three plants of the Yellow Eye variety. Eleven large chromosome pairs can be seen in the equatorial plates in the pollen mother cells, some of these showing clearly their bivalent nature (61-64). The chromosomes are arranged on the spindle in a fairly regular plate, and those of two pairs have a distinct crescent shape, by which they can be identified without much difficulty both in polar (figs. 61-63) and in lateral view (fig. 64). One of these two pairs is somewhat larger than the other. In the anaphases, some of the chromosomes show clearly the split preparatory for the homoeotypic division (figs. 65, 66). The sharply curved nature of two of the chromosomes is also noticeable.

The writer wishes to express his appreciation to Dr. C. E. Allen, under whose direction the work was carried on, and to whom he is greatly indebted for advice and criticism.

#### SUMMARY

1. The archesporium of the anther is a multicellular column, of from 3 to 5 cells in cross section. The outermost cells of the archesporium cut off the primary parietal cells, which form a layer about 3 cells in thickness.
2. The tapetum begins to differentiate when the heterotypic prophasees are well advanced.
3. A double spireme is formed during the early heterotypic prophasees of the pollen mother cells.
4. The pollen mother cells become rounded prior to diakinesis, and appear to be separated from one another by a thick gelatinous substance.
5. By the time meiosis is completed, the pollen mother cells have become surrounded by very thick walls. These walls seem to be homogeneous, and it is often difficult to distinguish a demarcation between adjoining cells.
6. Tetrad-formation begins with a pinching-in of the cytoplasm from the periphery of the mother cell, and a decreasing density of the cytoplasm along the path of cleavage. Thick partition walls are formed which seem to be continuations of the surrounding mother cell wall, and the young microspores appear to be set in a homogeneous matrix.
7. The matrix disintegrates very quickly, and a thick permanent wall is formed around each pollen grain.
8. A considerable proportion of the pollen grains show a tendency to germinate within the anther. Whether such germinated pollen grains play any part in fertilization has not been ascertained.
9. Usually a single macrospore mother cell is differentiated in the axial row of the nucellus. This takes place before the primordia of the integuments appear.
10. By two successive divisions a linear triad of macrospores is formed, the chalazal one of which develops into a 7-celled embryo sac of the usual type.

11. The antipodals are ephemeral, and the synergids also usually disintegrate before fertilization, so that the mature embryo sac commonly consists of two cells only—the egg and the primary endosperm cell.

12. The micropylar part of the nucellus is destroyed in the development of the macrogametophyte, but the larger chalazal portion persists until the embryo has reached a very advanced stage. The epidermal layer of the nucellus also persists for some time, and arches over the apex of the embryo sac.

13. Fertilization occurs (in the greenhouse) a few hours after pollination, temperature apparently playing an important part in the rapidity of the process.

14. The zygote by two transverse divisions forms a filament of three cells. The terminal cell of this filament develops into the embryo; the two basal cells form the suspensor.

15. There is no distinct separation between the embryo proper and the suspensor, the basal cells of which become rounded and swollen. The swollen cell at the base of the suspensor becomes multinucleate.

16. The primary endosperm nucleus divides before the zygote nucleus. The succeeding divisions of the endosperm nuclei are rapid but not simultaneous, and a multinucleate layer of endosperm is soon formed around the periphery of the embryo sac.

17. Cell division in the endosperm begins at about the time when the dermatogen is differentiated, and occurs only in the region in immediate contact with the embryo.

18. The endosperm is entirely absorbed by the time the embryo is mature.

19. The diploid chromosome number in *P. vulgaris* is 22.

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### EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida. Since different optical combinations were used for the various drawings, the magnification is given for each figure.

#### PLATE XVI

FIG. 1. Longitudinal section of a young anther sac, showing the differentiated archesporium and the formation of parietal tissue.  $\times 1150$ .

FIG. 2. Anther sacs in cross section, showing microspore mother cells.  $\times 1150$ .

FIG. 3. Microspore mother cells at the time of diakinesis, with the surrounding gelatinous matrix. Tapetum differentiated.  $\times 1150$ .

FIG. 4. A microspore mother cell; nucleus in resting condition.  $\times 2350$ .

FIGS. 5, 6. Early heterotypic prophase.  $\times 2350$ .

FIGS. 7, 8. Contraction of spireme to form synizetic knot.  $\times 2350$ .

FIG. 9. Loose spireme after synizesis.  $\times 2350$ .

FIG. 10. Thickening of spireme before segmentation.  $\times 2350$ .

FIG. 11. Segmentation of the thickened spireme.  $\times 2350$ .

FIGS. 12, 13. Diakinesis.  $\times 2350$ .

FIG. 14. Nuclei of a microspore mother cell during interkinesis. Chromosomes are anastomosed.  $\times 2350$ .

FIGS. 15, 16. Pollen germinated within the anther. Drawn from living material.  $\times 440$ .



## PLATE XVII

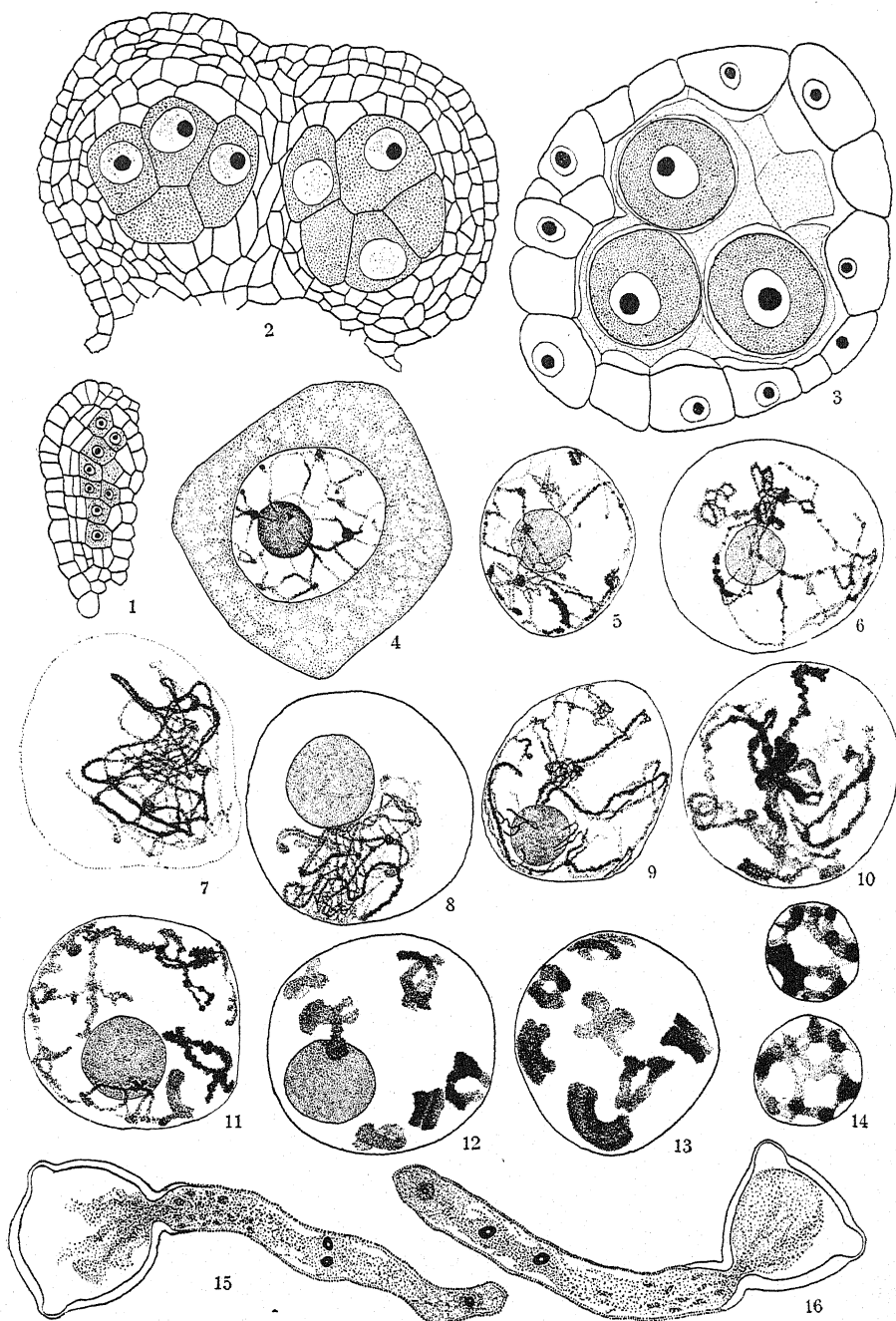
- FIG. 17. Tetranucleate microspore mother cell, showing thick wall.  $\times 1550$ .  
FIG. 18. Beginning of pollen-tetrad formation.  $\times 1550$ .  
FIG. 19. Pollen tetrad.  $\times 1550$ .  
FIG. 20. Young microspore.  $\times 1550$ .  
FIG. 21. Mature pollen grain with large tube nucleus in center and smaller generative cell to one side. Two of the three germ pores are shown.  $\times 1550$ .  
FIG. 22. Portion of a pollen tube with two male nuclei, as found in the basal end of the ovary.  $\times 1350$ .  
FIG. 23. Primordia of integuments. The ovule is at the stage of development shown in text figure 1.  $\times 600$ .  
FIGS. 24, 25. Stages in the development of the integuments and in the curving of the nucellus.  $\times 100$ .  
FIG. 26. Young macrospore mother cell.  $\times 440$ .  
FIG. 27. Older macrospore mother cell.  $\times 440$ .  
FIG. 28. Synizesis in the macrospore mother cell.  $\times 440$ .  
FIG. 29. Daughter cells of the macrospore mother cell during the homoeotypic division. Abortive spindle in the micropylar daughter cell.  $\times 440$ .  
FIG. 30. Typical linear triad of macrospores, the chalazal one beginning to enlarge.  $\times 440$ .  
FIG. 31. The nucleus of the functional macrospore has divided. Nuclear division has also occurred in the central macrospore, this being unusual.  $\times 440$ .  
FIGS. 32-34. Stages in the development of the embryo sac.  $\times 440$ .

## PLATE XVIII

- FIG. 35. Fully formed embryo sac.  $\times 440$ .  
FIG. 36. Mature embryo sac, showing the egg, the primary endosperm cell, and the remains of one synergid.  $\times 300$ .  
FIG. 37. Embryo sac showing the pollen tube, still containing the male nuclei, lying in close proximity to the egg. The tube nucleus shows very faintly, having been cut in sectioning. The polar nuclei are in process of fusion.  $\times 440$ .  
FIGS. 38, 39. Stages in fertilization. One male nucleus is fusing with the egg nucleus, the other male nucleus with one of the polar nuclei. The tube nucleus remains in the pollen tube.  $\times 440$ .  
FIG. 40. Two small nuclei are apparently fusing with one of the polar nuclei. The tube nucleus is missing from the pollen tube.  $\times 440$ .  
FIG. 41. The two polar nuclei have united before fertilization.  $\times 440$ .  
FIG. 42. The primary endosperm nucleus is dividing, while the fusion of egg and male nuclei is still incomplete.  $\times 440$ .  
FIG. 43. Early prophase in the division of the primary endosperm nucleus.  $\times 1350$ .  
FIG. 44. Same as figure 43. A, B, and C are parts of the same nucleus cut in sectioning.  $\times 2350$ .

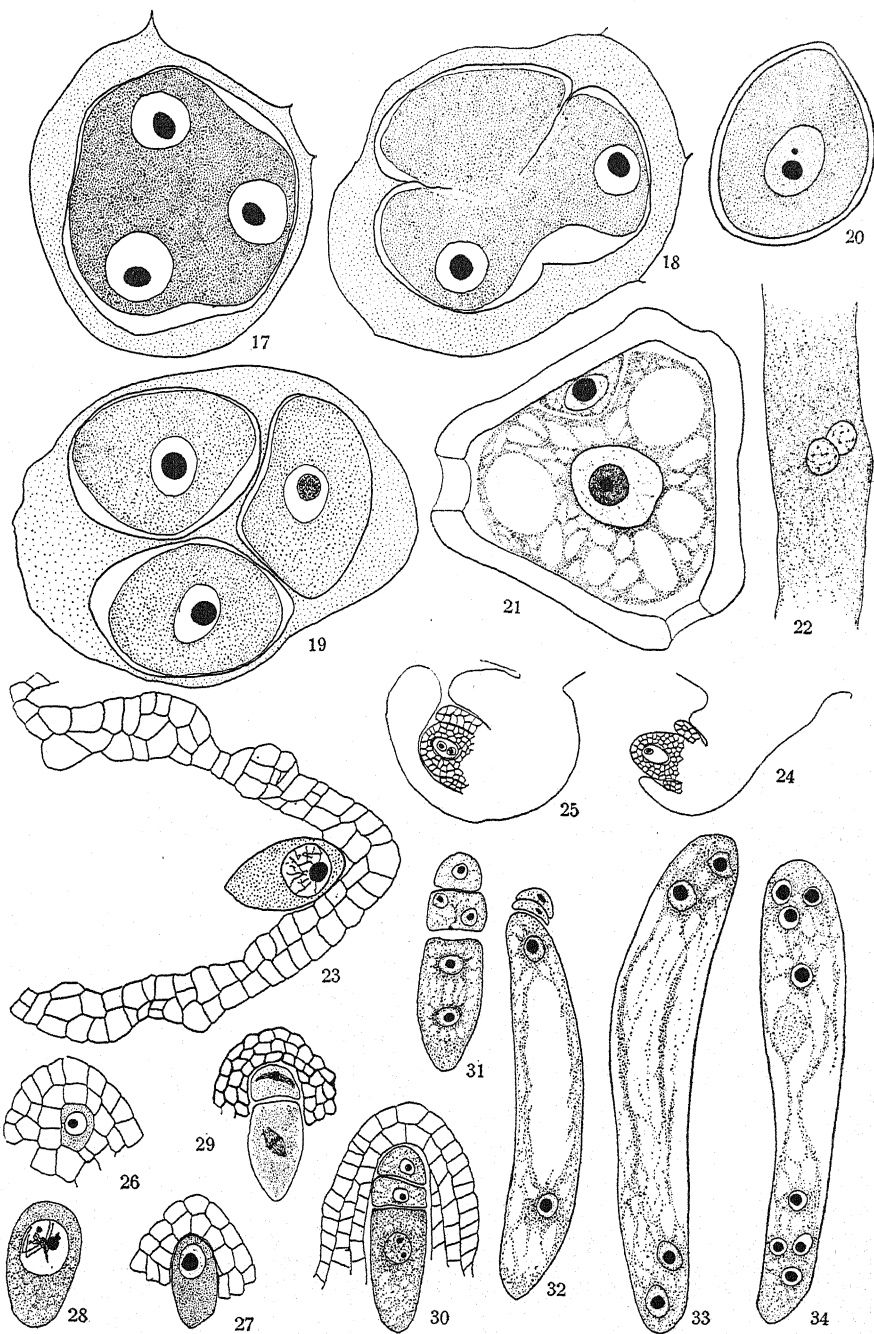
## PLATE XIX

- FIG. 45. A, B, C. Same as figure 44, Plate XVIII.  $\times 1350$ .  
FIG. 46. Chromosomes in the equatorial plate, division of the primary endosperm nucleus. A and B are parts of the same spindle cut in sectioning.  $\times 2350$ .  
FIG. 47. Telophase of first division of the zygote nucleus.  $\times 440$ .  
FIG. 48. Binucleate pro-embryo.  $\times 440$ .  
FIG. 49. A 2-celled pro-embryo. Nuclear divisions in endosperm.  $\times 440$ .  
FIG. 50. A 3-celled pro-embryo.  $\times 440$ .  
FIG. 51. A 4-celled embryo. The terminal cell has divided longitudinally.  $\times 440$ .



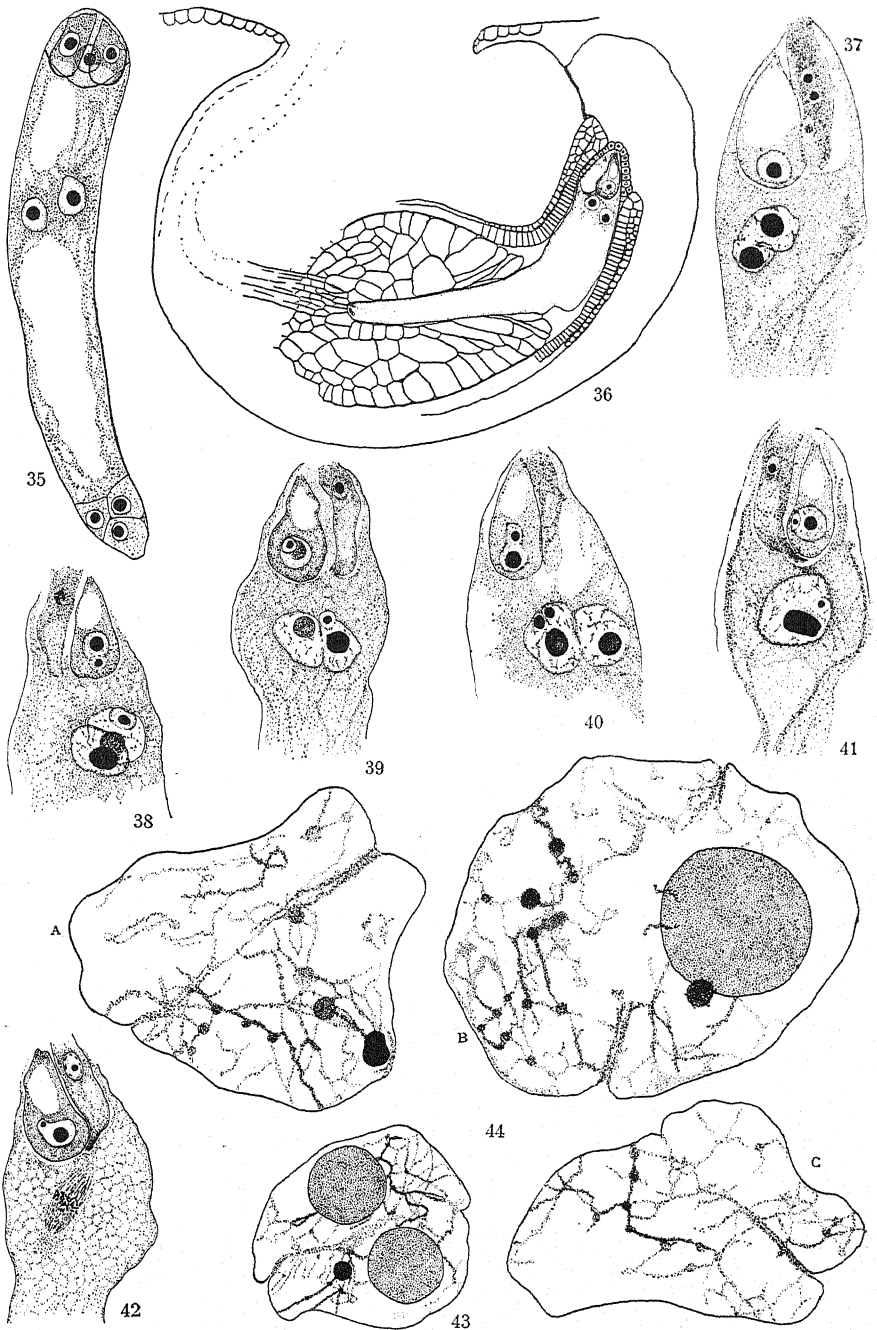
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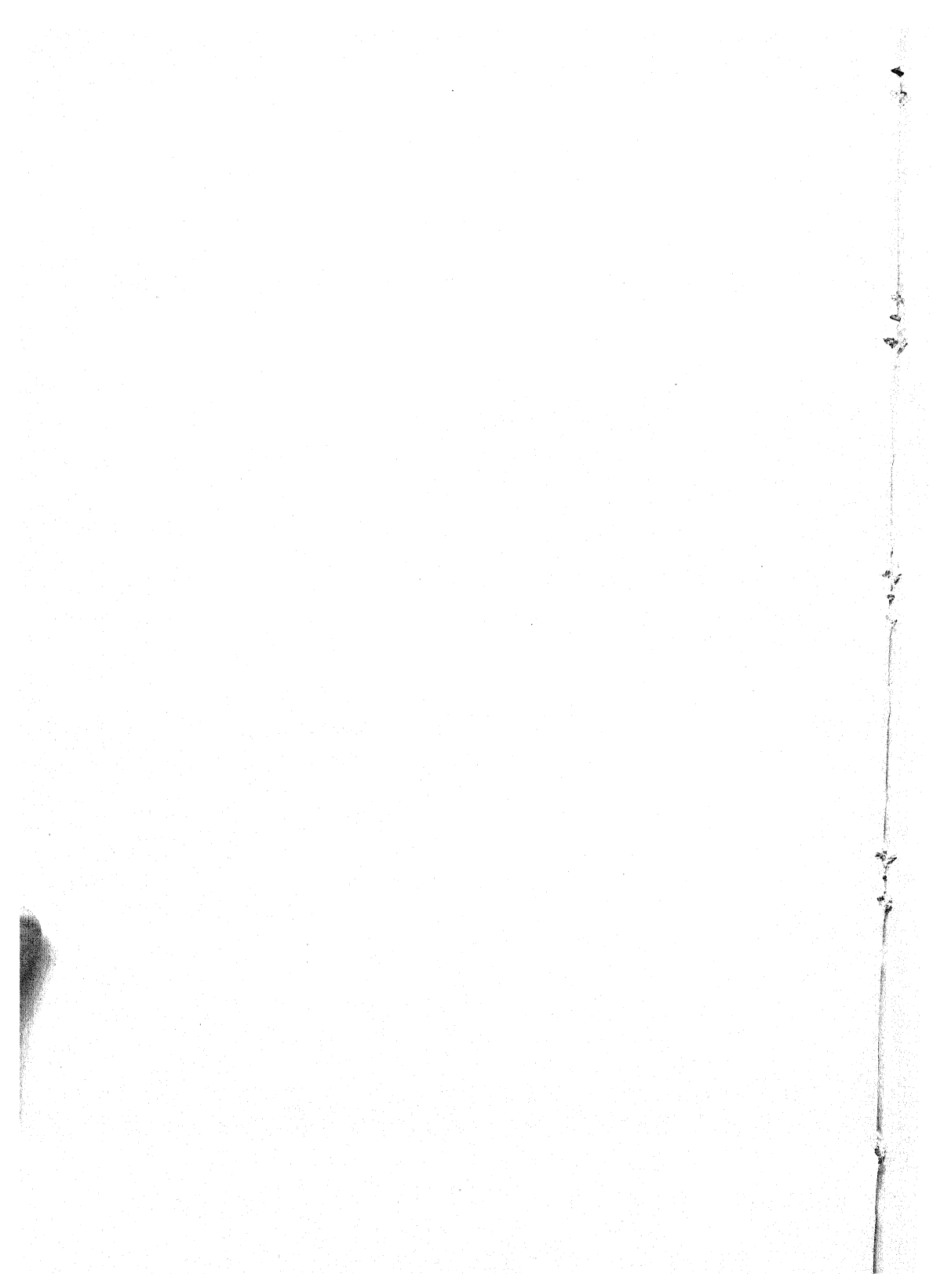


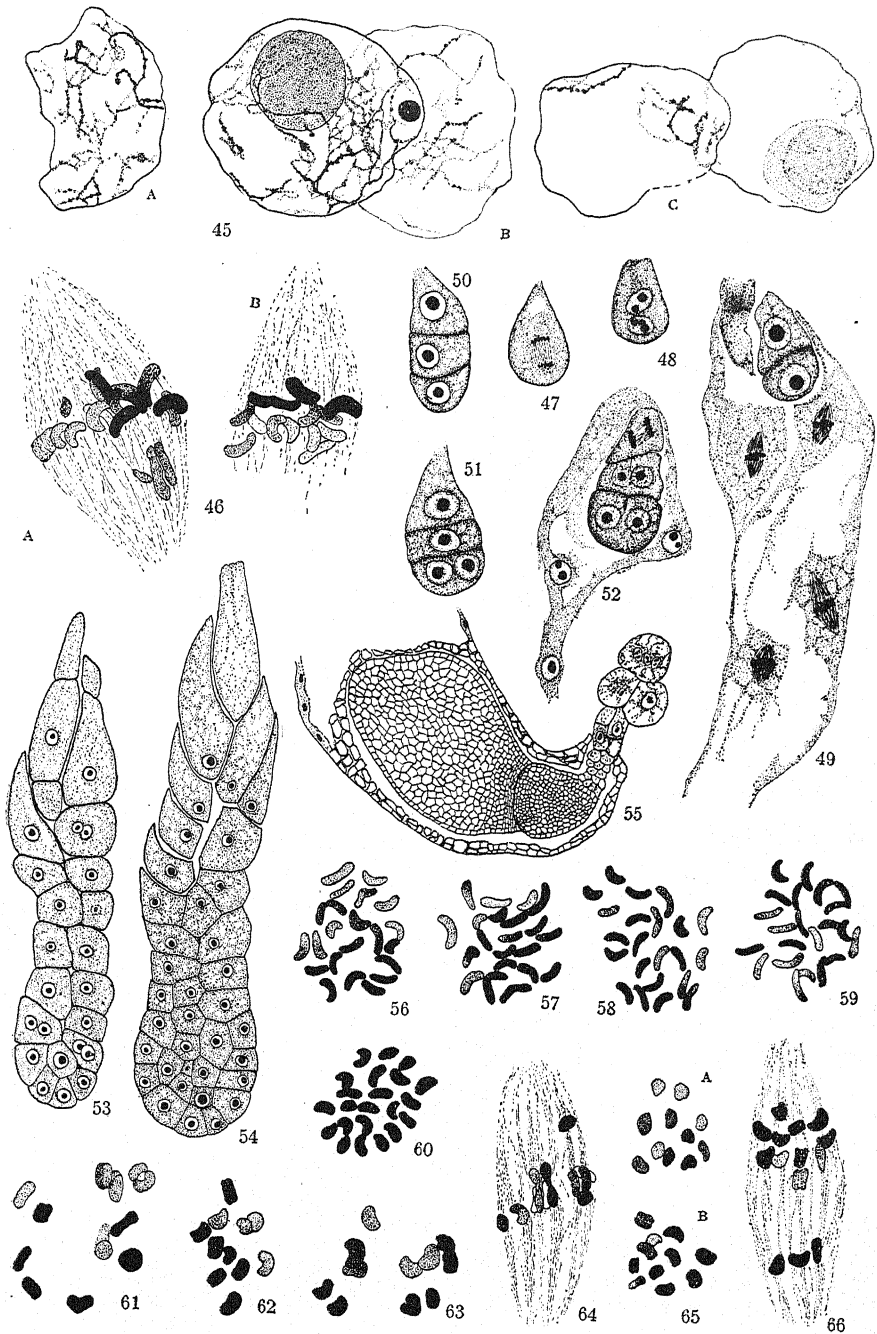


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WEINSTEIN: CYTOLOGY OF PHASEOLUS



1-1-1

FIG. 52. A 6-celled embryo, surrounded by endosperm.  $\times 440$ .

FIG. 53. Embryo at the beginning of dermatogen-differentiation.  $\times 440$ .

FIG. 54. An older embryo, showing no distinct demarcation between suspensor and embryo proper.  $\times 440$ .

FIG. 55. An embryo 11 days after fertilization, showing one cotyledon, the epicotyl, and the swollen cells of the suspensor. The rounded basal cell of the suspensor is multinucleate.  $\times 35$ .

FIGS. 56-59. Equatorial plates in cells of root tips. Polar view.  $\times 2350$ .

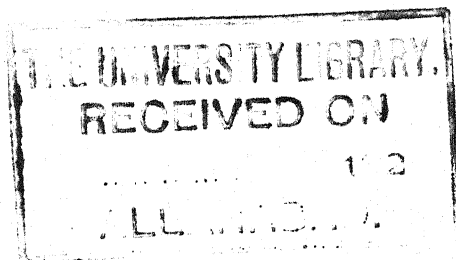
FIG. 60. Equatorial plate in cell of developing petal.  $\times 2350$ .

FIGS. 61-63. Polar views of chromosomes in heterotypic equatorial plates, microspore mother cells.  $\times 2350$ .

FIG. 64. Lateral view of heterotypic spindle, equatorial-plate stage.  $\times 2350$ .

FIG. 65, A, B. Polar views of sister chromosome groups in the heterotypic anaphases.  $\times 2350$ .

FIG. 66. Anaphase of heterotypic division. The lower chromosome group was cut in sectioning.  $\times 2350$ .





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## THE HYDROGEN-ION CONCENTRATION AND THE STAINING OF SECTIONS OF PLANT TISSUE

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One of the factors which influence the staining of tissues is that of acidity or alkalinity. Several investigators have observed the favorable effect of an acid reaction upon the uptake of certain acid dyes and its inhibitory effect upon the absorption of basic dyes by both plant and animal cells.

Bethe (1) found that preparations of the spinal cord when stained with toluidin blue were destained only when the destaining solution contained a surplus of hydrogen ions. In a later paper (2) he studied the dialysis relations of a number of stains to solutions of varying hydrogen-ion concentrations and concluded that cell content is a determining factor in the penetration of stains, and that the results obtained with living cells support the theory that the hydrogen-ion concentration is a factor in vital staining.

Robertson (11) observed that 0.01 *N* hydrochloric acid favored the staining of fat cells, connective-tissue cells, and red blood corpuscles by acid dyes, and that 0.01 *N* sodium hydroxid inhibited the staining. A similar result for basic dyes was secured with 0.01 *N* sodium hydroxid.

Harvey (5) found that the leaf cells of *Elodea* stained with a basic dye (neutral red) under alkaline conditions but did not absorb the same dye in the presence of a trace of acid in the medium. Filaments of *Spirogyra* were also used as well as certain animal cells such as sea urchin and starfish eggs and *Paramoecium*. Harvey concluded that basic dyes do not enter cells from an acid solution, and also that acid dyes are absorbed by cells from an acid solution but not from neutral or alkaline solutions.

Rohde (12), working with living tissues, concluded that acid and basic dyes probably enter all cells, but can be observed only in those in which the reaction of the interior of the cell is favorable to the accumulation of the dyes. Basic dyes were very rapidly absorbed by cells of basic reaction and acid dyes by slightly acid cells.

Collander (4) made a rather detailed study of the absorption of a large number of the sulfonic acid dyes with a wide variety of living plants and concluded that the absorption of these acid dyes was dependent in high

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degree upon the hydrogen-ion concentration of the solution. Hydrogen ions favored the uptake of these dyes and hydroxyl ions hindered such absorption.

These observations on the absorption of dyes under acid and alkaline conditions indicate that plant tissues act much like a protein. Loeb (7) has shown that a pure protein combines with acid dyes under acid conditions and with basic dyes under alkaline reactions. This was demonstrated by bringing different doses of powdered gelatin to different pH values and treating them with an acid dye such as acid fuchsin. After thorough washing with cold distilled water it was found that only those lots of gelatin were red whose pH values were less than 4.7. All above pH 4.7 were colorless. Basic dyes combined with gelatin only when the pH value was greater than 4.7. In other words, the hydrogen-ion concentration determined the staining of gelatin with acid and basic dyes with pH 4.7 as a critical concentration. This point is the isoelectric point for gelatin.

Thomas and Kelly (13), using the dye method with collagen from hide powder, found that their results did not reveal a sharp isoelectric point such as was found by Loeb for pure gelatin. They concluded that collagen is a mixture of proteins, and the isoelectric point for this material was placed at pH 5.0.

Robbins (9) used the dye technique in the determination of the isoelectric point for various plant tissues. With potato-tuber tissue the response to the acid and basic dyes used was much as one would expect of a protein with an isoelectric point between pH 6.1 and pH 6.95. An acid dye like eosin was held by the potato-tuber tissue from buffer mixtures whose pH value was 6.1 or less, and was held weakly by the tissue from buffer mixtures whose pH values were 6.95 or above. Results obtained by Robbins (10) with the mycelia of *Rhizopus nigricans* and *Fusarium lycopersici* also indicated that both the fungi used responded to acid and basic dyes much as does a protein with an isoelectric point. *Rhizopus nigricans* responded to the dyes as if that point were in the vicinity of pH 5.0, and *Fusarium lycopersici* responded as if that point were near pH 5.5.

While the brief review of literature here given indicates the importance of hydrogen-ion concentration in determining the staining of plant tissues and shows that their response is in general much like that of a protein, comparatively little attention has been paid to its influence in general cytological or histological technique. Lee (6) states that the stain given by acid dyes is fast to acids and may be intensified by them; while basic dyes are washed out by acids but intensified by alkalis. Chamberlain (3), aside from the recommendation of acid alcohol for destaining, makes no reference to the importance of reaction. It was thought advisable, therefore, to investigate the effect of hydrogen-ion concentration on the staining of plant tissues using the common methods of cytological technique. The investigation was made in 1923 (8) at the suggestion of Dr. W. J. Robbins

and following preliminary experiments performed by him in 1922 on the effect of reaction on the staining of sections of root tips and of bacteria.

### METHODS AND MATERIALS

The plant tissues used were root-tip tissues of hyacinth, buckwheat, lupine, pea, and soybean. The roots were fixed in chrom-acetic acid, dehydrated in alcohol, run into chloroform, and finally imbedded in paraffin and sectioned.

The acid dyes used were: eosin, yellowish, Will Corporation (no. 512);<sup>1</sup> methyl blue, Grübler (no. 478); Martius yellow, Bausch and Lomb (no. 3); and orange G, Bausch and Lomb (no. 14). The basic dyes used were: malachite green, Bausch and Lomb (no. 427); toluidin blue, Grübler (no. 654); safranin, Grübler (no. 584); and basic fuchsin, Will Corporation (no. 448). These dyes were used in 1% aqueous solutions in most cases, and the period of staining varied from 1 or 2 to 10 or 15 minutes. Buffer mixtures were made by titrating 50 cc. of  $M/10$   $H_3PO_4$  with amounts of  $N/10$  NaOH sufficient to produce a series of buffer mixtures which ranged by about 0.5 Sorensen unit from near pH 3.5 to somewhat above pH 7.0. These stock solutions were diluted 100 times with redistilled water, and the pH values were determined by Gillespie's colorimetric method.

The general procedure followed for the experiments described here was briefly as follows: Sections of the material imbedded in paraffin were mounted on slides, treated with xylol to remove the paraffin, and transferred through alcohol of various dilutions to redistilled water. The slides bearing the sections were placed in about 100-cc. quantities of the phosphate buffer mixtures of different H-ion concentrations and allowed to stand a few minutes. Ordinary staining jars were used as containers. The slides were then removed and the sections were stained for from 5 to 10 minutes with an aqueous solution of the dye selected. Washing then followed in the same buffer mixtures by simply allowing the slides to stand for a period, in most cases, of 6 hours with a complete change of the buffer mixtures every 2 hours. The sections were then dehydrated rather rapidly in alcohol, cleared, and mounted in balsam. Observations were made macroscopically upon the effect of washing at the various reactions on the loss or retention of the dye by the tissue as a whole, and microscopically on the loss or retention of the dye by parts of the cell.

### RESPONSE TO ACID DYES

Martius yellow and orange G proved unsatisfactory because of the rapidity with which these dyes were lost by the tissues during washing and dehydration. With methyl blue and eosin, the tissues listed above showed macroscopically a response much like that of a protein with an isoelectric point lying between pH 5.3 and pH 6.1. These two acid dyes were retained

<sup>1</sup> The numbers are the Schultz numbers of the dyes used as given by A. G. Green. A systematic survey of the coloring matters, 2d ed. London, 1904.

strongly by the tissues when washed by solutions of pH 3.5 to pH 5.0, and largely or entirely lost in solutions of pH 5.6 and above.

Microscopic examination showed that all parts of the cell did not act alike. The cytoplasm lost the acid dyes at more acid reactions than did the chromatin of the resting nucleus. The chromosomes and nucleoli, however, retained the dyes in sections washed in solutions of reactions at which the cytoplasm and chromatin of the resting nucleus had lost almost all stain. The cytoplasm appeared to have an isoelectric point more acid than that of the chromatin material. The chromosomes and the nucleoli responded in their retention of acid dyes like proteins with isoelectric points distinctly more alkaline than that of the chromatin of the resting nucleus. Microscopic examination also revealed that all the cells did not act exactly alike. Occasional cells here and there, especially in the region of the central cylinder, retained the dye at more alkaline reactions than did cells in other regions of the root tip.

The following is a record of a typical experiment showing the response of root-tip tissue to the acid dye, eosin. Ten slides were selected on each of which were several entire sections of hyacinth root tips. These slides were placed in xylol for 10 minutes and then transferred through various dilutions of alcohols to redistilled water. They were then placed in a series of phosphate buffer mixtures for a few minutes, taken out, drained, and immersed in a 1-percent aqueous solution of eosin for 10 minutes. They were washed with redistilled water until the excess stain was removed, and replaced in the buffer mixtures. The slides were washed for 6 hours by allowing them to stand in the solutions, occasionally moving them back and forth, and changing the mixtures completely every 2 hours. At the end of the washing period they were dehydrated with alcohol, cleared in xylol, and mounted in balsam.

The sections after washing and mounting showed a gradation in color from a bright red on the acid end of the series to a faint pink on the alkaline end. Sections washed with buffer mixtures with pH values of 3.5, 4.1, and 4.4 retained the stain strongly and were bright red in color. Those washed with buffer mixtures with pH values of 5.4 and 5.9 retained the stain less strongly; and those on the last 5 slides, washed with buffer mixtures with pH values of 6.5, 6.9, 7.7, 8.3, and 8.8, lost nearly all color. These sections were scarcely visible when held over a white background.

Microscopic observation showed that in sections on the first two slides, washed with buffer mixtures with pH values of 3.5 and 4.1, the cytoplasm stained a light pink, the chromatin pink, and the nucleoli a bright pink. The cytoplasm became quite pale in color in sections washed with buffer mixtures with pH values of 4.4 and 5.4, and lost all color in sections washed with the buffer mixtures with pH values of 6.5 and above. The chromatin of the resting nucleus also lost much of its color at this point, and only the chromosomes and the nucleoli stood out in the sections washed with buffer

mixtures with pH values above 6.5. Very little, if any, color could be detected in the sections on the last 3 slides washed with buffer mixtures with pH values of 7.7, 8.3, and 8.8. In general, the cytoplasm and cell walls lost the acid dye first, then the chromatin of the resting nucleus, and the last parts of the cell to lose the dye toward the alkaline end of the series were the nucleoli and the chromosomes.

#### RESPONSE TO A BASIC DYE

Employing the same general technique as described above, the basic dye, malachite green, was used with root tips of hyacinth and soybean. It was retained by the tissues in solutions of alkaline reactions and lost in solutions of acid reactions, or just the reverse of the results secured with the acid dye, eosin. The blue color was entirely lost in the first 4 slides, or in sections washed with buffer mixtures of pH 5.0 and below. Sections on slide number 5, held at a pH value of 5.5, were but slightly blue, while all the sections washed with buffer mixtures with pH values of 6.1 and above held the color with almost equal intensity. Satisfactory microscopic examinations of these sections could not be made because of the loss of considerable of the stain in the dehydration with alcohol.

#### RESPONSE TO ACID AND BASIC DYES USED TOGETHER

The experiments with single acid and single basic dyes showed that an acid dye was held by sections of the root tips used when washed in acid solutions and lost at more alkaline reactions, and that the basic dye was retained in alkaline solutions and lost in more acid ones. This suggested that both acid and basic dyes might be used at the same time, in which case both dyes should appear in the same series; that is, sections at the one end of the series staining with the acid dye and those at the other end staining with the basic dye. This would be similar to the procedure used and the results found by Robbins (10) with methylene blue and eosin for the mycelia of fungi.

Eosin and toluidin blue were selected as the two stains because they gave a sharp color contrast. Six experiments were performed with this combination of dyes upon tissues of 5 different plants. The general response was much like that of a protein with an isoelectric point lying between pH 4.3 and pH 5.0. In this critical range, however, both dyes were retained by the tissues, a reaction which would not be expected of a pure protein judging from the results secured by Loeb (7). When sections of root tips were stained with eosin and then with toluidin blue they appeared purplish. Upon washing the doubly stained sections in buffer solutions of pH 3.5 to pH 7.3, the toluidin blue came out of the sections washed in the acid solutions (pH 3.5 to 4.3), leaving the sections red; while in the more alkaline solutions (pH 5.0 to 7.3) the eosin came out, leaving the sections blue. At the intermediate reactions, pH 4.6 to pH 5.0, both the acid and



the basic dye were retained, the sections appearing purplish on macroscopic examination. In this region differential staining was secured.

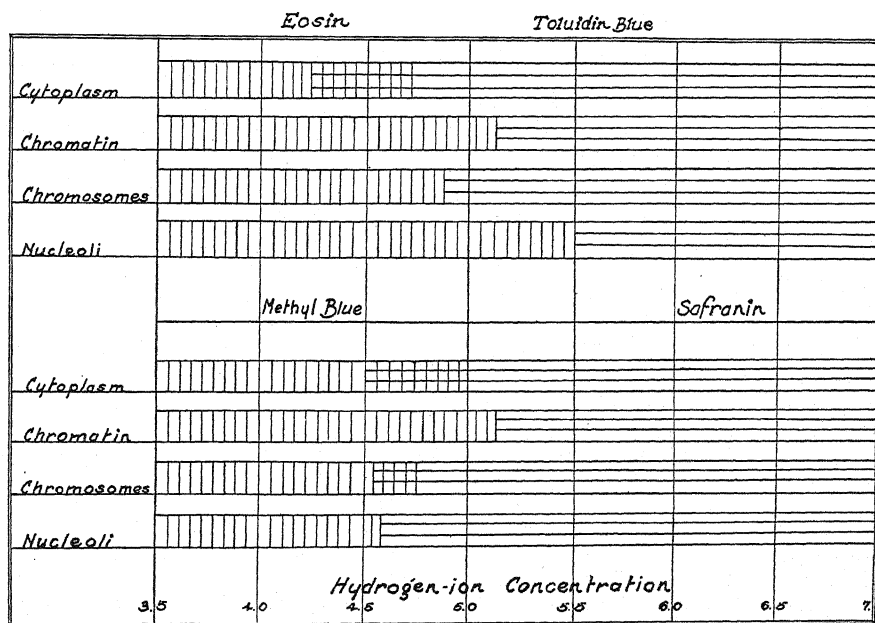
The following-described experiment will illustrate. Root-tip tissues of hyacinth were treated as already described except for the details of staining. In this case two dyes were used instead of one. All the sections were stained at the same time for a period of 10 minutes in the acid dye (eosin), washed with redistilled water until the stain ceased to come out in clouds, and again stained for a period of 10 minutes in the basic dye (toluidin blue). After the staining and the removal of the excess of stain with redistilled water the sections were of a fairly uniform purple color, indicating that both stains had been absorbed. The sections were then washed in the buffer mixtures, the washing period being 6 hours with a change of the mixtures every 2 hours.

After dehydrating and mounting in balsam, the series of sections showed macroscopically a range in colors from red at one end through intermediate colors to blue at the opposite end of the series. Sections washed with buffer mixtures with pH values of 3.5 and 3.9 were red. The sections washed in the solution of pH 4.3 were purplish with considerable red. Those washed with buffer mixtures with pH values of 4.6 and 5.0 were purple. The sections washed with buffer mixtures of pH values higher than 5.0 were blue. In Plate XX the final colors of the sections as viewed macroscopically against a white background are represented.

Under the microscope the cells of sections on the first 3 slides, washed in solutions at pH 3.5, 3.9, and 4.3, were found rather uniformly stained with the eosin. The cell walls and cytoplasm stained pink, while the chromatin and the nucleoli stained somewhat brighter pink. Sections on slides nos. 4 and 5, washed with solutions at pH 4.6 and 5.0, held both the dyes. The cell walls and cytoplasm were blue and the chromatin of the resting nucleus and the nucleoli were red, the latter being bright red. When dividing cells were found in sections washed at pH 5.0, the chromatin during the prophase was red, and later in the metaphases and anaphases the chromosomes took the blue stain. In still later stages involving the reconstruction of the daughter nuclei the chromatin again appeared red and the spindle blue, especially across the center where the fibers were beginning to thicken. The nucleoli upon reappearance took the red stain as did also the chromatin, as described for the resting nucleus. In the sections on the last 5 slides, washed with solutions with pH values higher than 5.0, all parts of the cell stained equally blue, except the nucleoli which still showed a faint red at pH 5.6. The retention of these two dyes by the various parts of the cell is illustrated in text figure 1.

This combination of stains in several experiments showed that the root tips of pea, hyacinth, lupine, soybean, and buckwheat behaved in a very similar manner. The acid dye was taken up and held only in acid reactions, and the basic dye was held only in solutions which were more alkaline.

Between these two regions the retention of the dyes overlapped. This intermediate region is narrow, lying between pH 4.6 and pH 5.0. Within this range some parts of the cell held the acid stain and other parts retained the basic stain, so that macroscopically the sections had an intermediate color between red and blue, *i.e.*, purple. The cytoplasm took up the blue basic stain strongly under alkaline conditions and retained it into acid reactions where the nucleus had lost the toluidin blue and was stained with the eosin. In this region where the absorption of acid and that of basic dyes overlap, pH 4.6 to 5.0, differential staining was secured.



TEXT FIG. 1. The limits of retention of the acid dyes, eosin and methyl blue, and the basic dyes, toluidin blue and safranin, by the parts of the cell in sections of root tips of hyacinth. Sections stained with both acid and basic stains and washed with dilute buffer mixtures of different reactions.

After using the eosin and toluidin blue combination with different tissues, another combination of stains was tried with the same tissues. Instead of using an acid stain which was red and a basic stain which was blue, the situation was reversed. Methyl blue was selected as a blue acid stain and safranin as a red basic stain. The method of staining and washing was the same as that already described. A typical experiment with hyacinth root tips is here described.

After washing, dehydrating, and mounting in balsam, the series of slides showed colors opposite to those of the previous experiment. The blue color was held at the acid end and the red color at the alkaline end of

the series. Sections on slides 1, 2, and 3 washed with buffer mixtures with pH values of 3.5, 3.9, and 4.3 were quite blue; sections on slides 4 and 5 washed with buffer mixtures with pH values of 4.6 and 5.0 were purplish; and all sections washed with buffer mixtures with pH values higher than 5.0 were red. The final colors of all the sections as viewed macroscopically are represented in Plate XX. The response under varying H-ion concentrations was much like that of a protein with an isoelectric point lying between pH 4.6 and pH 5.0.

Microscopic examination revealed in sections washed with buffer mixtures with pH values of 3.5, 3.9, and 4.3 that all parts of the cell were stained blue with the acid stain. The chromatin took the blue color most deeply, the cytoplasm and cell walls to a lesser degree, while the nucleoli held the least color. The chromosomes stained about like the chromatin of the resting nucleus. In slides 4 and 5, washed with buffer mixtures with pH values of 4.6 and 5.0, the chromatin of the resting nucleus stained blue, the nucleoli slightly pink, and the cytoplasm and cell wall purplish with the red predominating. In dividing cells in these sections the spireme thread stained red with the basic stain, as did likewise the chromosomes as soon as they were formed. The spindle retained the blue acid stain, especially toward the center, or in that portion lying between the two groups of daughter chromosomes. In late anaphase stages the contrast between the red chromosomes and the blue spindle gave good differential staining. In telophase stages the spireme thread again held the red basic stain about as did the chromosomes, and later, when the daughter nuclei were formed, the chromatin once more stained blue with the acid dye. In Plate XX camera lucida drawings of cells taken from hyacinth root tips show how methyl blue and safranin were retained by the cell parts during the process of nuclear and cell division when the sections were washed with buffer mixtures of pH 5.1. The colors are represented as closely as possible. In sections on slides washed with buffer mixtures with pH values above 5.2, all parts of the cell stained red. The response of the different parts of the cell to methyl blue and safranin is shown in text figure 1.

#### DISCUSSION

The experiments described show that the hydrogen-ion concentration has a marked influence upon the staining of dead plant tissues by acid and basic dyes. The acid dyes used were taken up and held strongly by root-tip tissues of pea, hyacinth, buckwheat, lupine, and soybean when washed in buffer mixtures with pH values of 3.5, 3.9, 4.3, and 4.6, but were held very weakly when washed in buffer mixtures with higher pH values. The basic dyes used were taken up and held strongly at pH values of 5.6 to 7.3, but were held very weakly in solutions of more acid reaction. The general response of the tissues was much like that of a protein with an isoelectric point between pH 4.3 and pH 5.0.

As a result of these experiments, the isoelectric point must be expressed as a range and not as a point for two reasons. Not all parts of the cell stain alike. When a single acid dye is used, such as eosin, microscopic examination shows that the cytoplasm and cell wall lose their color first as one passes from the acid end of the series toward the alkaline end. The chromatin of the resting nucleus then loses its color, followed by the chromosomes and the nucleoli. This would indicate that in these dead and fixed tissues the cytoplasm and cell wall have the most acid isoelectric points, near pH 4.3, followed by the chromatin of the resting nucleus, near pH 5.0, and that the chromosomes and the nucleoli have the most alkaline isoelectric points, pH 5.6 or above.

When both acid and basic dyes are used, somewhat different results are secured. The cell wall and cytoplasm again respond as though they had the most acid isoelectric points. The chromatin of the resting nucleus, however, with the combination of dyes used, appears to have a more alkaline isoelectric point than the chromosomes. The response of the nucleoli varies. With the eosin-toluidin blue combination their isoelectric point appears to be near pH 5.5, and with the methyl blue-safranin combination near pH 4.6. These variations may be due to differences in reactivity of the stains with the cell constituents, or to the fact that both stains are absorbed over a range and that one predominates because of its greater intensity. A second reason why the isoelectric point must be expressed as a range is that the individual cell parts when examined microscopically do not show a sharp division line between the acid- and the basic-dye absorption. This is particularly true of the cytoplasm, which retains both dyes over a considerable range (pH 4.6 to 5.0). This behavior probably indicates the presence of a mixture of proteins.

The plants from which root tips were secured are highly diverse in their habitat requirements. They include the acid-soil-resistant buckwheat and lupine and the calciphilous pea. A difference in response of the tissues of these plants might be expected. However, they all respond very much alike as far as these experiments show.

We obviously can not conclude from these experiments that living cells or cell parts, or tissues fixed by other methods, will respond in dye absorption as did this dead and fixed tissue. Death probably changes the character of the cell constituents, and the chromic acid used in the fixing solution also probably affects the chemical nature of the cell contents. The fact that all parts of the cell respond much like ampholytes with isoelectric points in the general range of pH 4.6 to pH 5.6 supports the generally accepted idea of the fundamental importance of proteins in the make-up of the protoplasm, and also suggests that proteinaceous material is included in the make-up of the young cell wall.

It is generally stated that the nucleus is basophile and the cytoplasm acidophile. These experiments show that the cytoplasm or nucleus can

be stained with either acid or basic dyes, depending upon the reaction of the solutions used in washing after staining. They also emphasize the importance of controlling reaction in the general technique of histology and cytology. They suggest that by controlling the reactions of washing solutions a new approach may be made upon the differentiation of cell parts one from another, and that thus additional information as to their chemical constitution may be secured.

#### SUMMARY

1. Sections of root tips of hyacinth, buckwheat, pea, soybean, and lupine, fixed in chrom-acetic acid, were stained with acid and basic dyes and washed in dilute buffer mixtures of phosphoric acid and sodium hydroxid. The acid dyes used were retained strongly by the tissues in solutions of acid reaction (pH 3.5-4.6) and were largely lost in solutions of a more alkaline reaction (pH 5.0-7.3), the greatest loss occurring in the most alkaline solutions. The basic dyes used were retained by the tissues in solutions of alkaline reaction (pH 5.6-7.3) and lost gradually in solutions of more acid reaction, the greatest loss occurring in the most acid solutions.

2. The plant tissues employed responded to acid and basic dyes under varying hydrogen-ion concentrations much as does a protein with an isoelectric point lying between pH 4.6 and pH 5.0.

3. The isoelectric point for the plant tissues used as defined by the dye method of determination was a range and not a point.

4. The root tips of all the plants used responded much alike in the retention of acid and basic dyes under varying hydrogen-ion concentrations.

5. Microscopic examinations showed that the parts of the cell had behaved differently toward acid and basic dyes under varying hydrogen-ion concentrations. The cytoplasm apparently has a more acid isoelectric point than do the various parts of the nucleus.

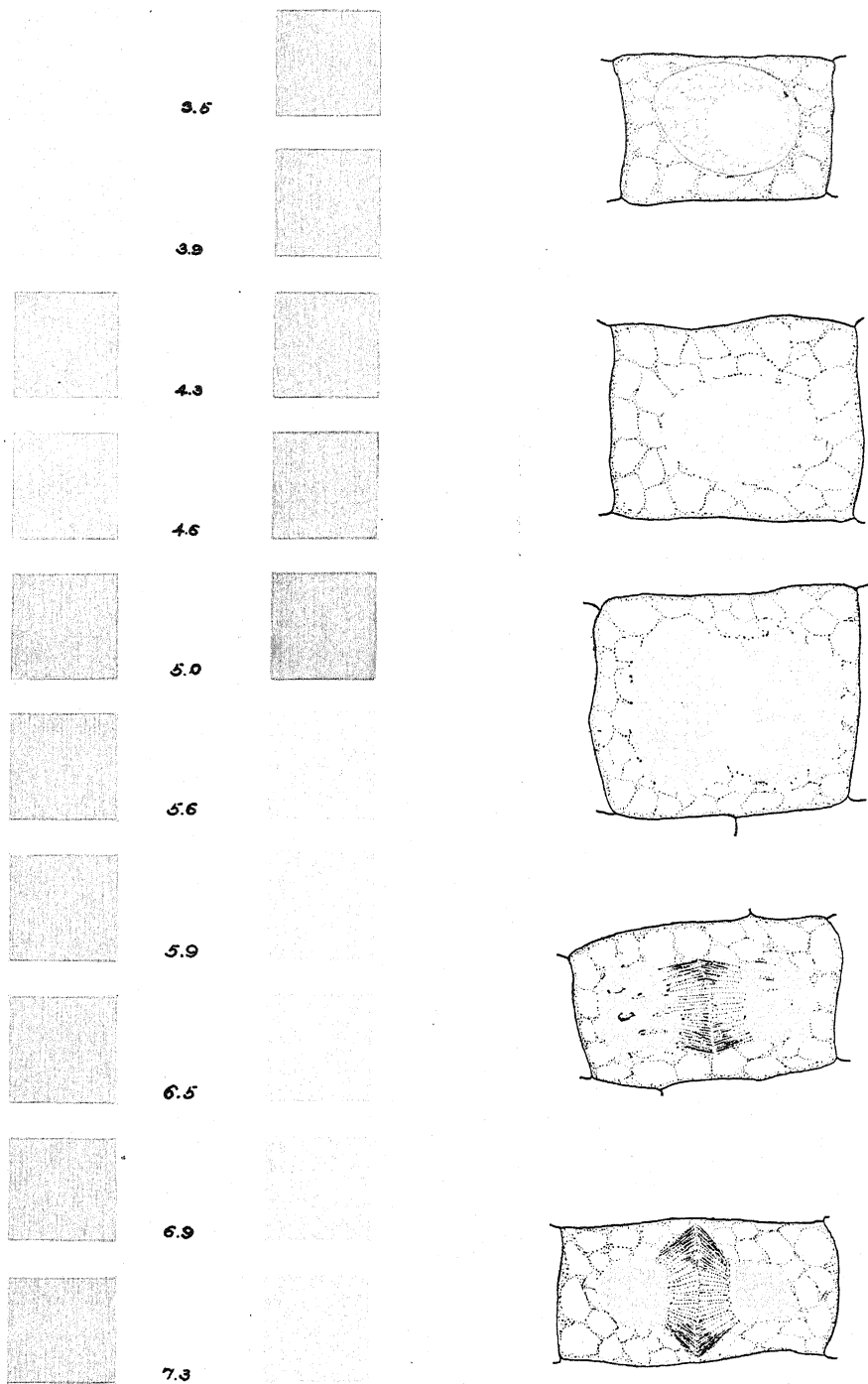
6. A new method of differential staining has been developed. By staining with an acid and a basic stain of contrasting colors and washing with buffer mixtures at a certain critical hydrogen-ion concentration, differences in the retention of the dye, by different parts of the cell result in differential staining.

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#### EXPLANATION OF PLATE XX

The squares represent the macroscopic appearance of sections of hyacinth root tips, stained with eosin and toluidin blue (left) and methyl blue and safranin (right), and washed with dilute buffer mixtures of the reactions indicated. The figures of cells show the differential staining secured in the cells of hyacinth root tips when stained with both methyl blue and safranin and washed with a buffer mixture of pH 5.1.



# CAN A PYRROLE DERIVATIVE BE SUBSTITUTED FOR IRON IN THE GROWTH OF PLANTS?

C. G. DEUBER

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In a preliminary note Pollacci and Oddo (4) discuss the presence of iron in the pigment of vertebrate blood and that of magnesium in the chlorophyll of green plants, and the fact that these metals are bound to organic complexes which yield similar pyrrole compounds on reduction. An experiment was described in which corn seedlings were grown in a nutrient solution lacking iron and in one containing a magnesium salt of pyrrole carbonic acid in place of iron. After 20 days' growth the corn plants in the solution containing the pyrrole salt were normally green and three times the size of the chlorotic plants grown without iron. In a more extensive paper (3) these investigators report normal growth and chlorophyll-development of 6 species of plants in solutions containing this pyrrole salt but lacking iron. From these experiments and from the analytical work of Willstätter (5, 6) upon the chemistry of chlorophyll the theory was advanced that iron functions as a catalyst in the formation of pyrrole groupings which go to make up the nucleus of the complex chlorophyll molecule. The Italian workers contend that, when the pyrrole group is supplied to plants in a form which can be used, iron becomes unnecessary for the formation of chlorophyll.

The writer became interested in this subject through efforts to induce chlorophyll-development in some genetically yellow corn strains. It was soon apparent that considerable work would have to be done to secure positive results with normally green plants with a pyrrole salt substituted for iron. The main object of the present investigation therefore became an attempt to confirm the work of Pollacci and Oddo.

The literature dealing with the physiological action of pyrrole compounds in relation to plants is very limited. Ciamician and Galizzi (1) report that pyrrole carboxylic acid and dimethyl-pyrrole dicarboxylic acid are not toxic to plants. R. Emerson of Harvard University, in a communication to the writer, states that 2-4-dimethyl-3-5-dicarbothoxy-pyrrole and 2-phenoxy-pyrrole have been used in nutrient solutions for the growth of several species of plants with varying success.

The magnesium salt of pyrrole carbonic acid used in these experiments, except for a small sample received from Prof. Oddo, was prepared by J. S. Chu<sup>1</sup> and the writer in the Organic Chemistry Laboratory of the

<sup>1</sup> The writer wishes to acknowledge the assistance of Mr. J. S. Chu in the synthesis of the pyrrole salt and for making the analysis given above.

University of Missouri. The methods of Oddo and Moschini (2) for the synthesis of  $\alpha$ -pyrrole carbonic acid were followed, and its magnesium salt was made according to the method of Oddo and Pollacci (3). The compound was purified by boiling with absolute alcohol. Melting-point determinations resulted in the compound's changing color and decomposing with the liberation of pyrrolic vapors between  $255^{\circ}$  and  $260^{\circ}$  C. Oddo and Pollacci state that the decomposition of the salt occurs at  $260^{\circ}$  C. in a closed tube.

An analysis of the compound was made with the following results:

	Found	Calculated
Mg. ....	9.81%	9.95%
N as $\text{NH}_3$ .....	10.26%	12.68%
Loss on ignition.....	83.98%	83.51%
Moisture.....	23.10%	14.77%

The magnesium was determined by ignition of the compound to magnesium oxid, conversion to magnesium sulfate with sulfuric acid, and the titration of the excess sulfuric acid with sodium hydroxid. The nitrogen was determined as ammonia by the Kjeldahl method, and the moisture content by heating in an oven at  $113^{\circ}$  C. The results of these determinations indicate agreement in the composition of the compound with the theoretical in all respects except moisture. The analysis shows the presence of 3 molecules of water, while Oddo and Pollacci report 2 molecules of water attached to the compound.

The cultural studies with this pyrrole salt were carried out as far as possible in the same manner as described by the Italian workers. The nutrient solutions employed by them were used, consisting of solution *A*, which lacked iron and the pyrrole salt, and solution *B*, lacking phosphorus and iron but containing the pyrrole salt. A solution *C* was also used which contained in addition to the salts of solution *A* an iron salt, in order to compare the efficiency of the pyrrole salt with iron, a comparison which the Italian workers have failed to make. The compositions of the solutions were as follows:

Solution A		Solution B	
$\text{Ca}(\text{NO}_3)_2$ .....	1.00 g.	$\text{Ca}(\text{NO}_3)_2$ .....	1.00 g.
$(\text{NH}_4)_2\text{SO}_4$ .....	0.25 g.	$(\text{NH}_4)_2\text{SO}_4$ .....	0.25 g.
$\text{KNO}_3$ .....	1.00 g.	$\text{KNO}_3$ .....	1.00 g.
$\text{KH}_2\text{PO}_4$ .....	0.25 g.	Pyrrole salt.....	
Dist. water.....	1,000 cc.	Dist. water.....	1,000 cc.

#### GROWTH OF REID'S YELLOW DENT CORN WITH THE MAGNESIUM SALT OF PYRROLE CARBONIC ACID SUBSTITUTED FOR IRON

Reid's Yellow Dent corn seedlings were grown in solution *A* minus iron, solution *B* with the pyrrole salt at the rate of 0.125 g. per liter, and in solution *C* containing 0.03 g. of ferric chlorid per liter. Since it was not understood why Oddo and Pollacci omitted phosphorus from the

nutrient solution containing the pyrrole salt, solution A plus 0.250 g. of the pyrrole salt was also used. The cultures were made in 500-cc. wide-mouth bottles wrapped in black paper and provided with paraffined paper tops. Two 5-day-old seedlings were placed in each culture.

After 2 weeks' growth the corn plants in the solutions containing the pyrrole salt were decidedly stunted in size. The leaves of these plants were a deep bluish green and the culms dark red, while the roots were discolored and the development of lateral roots was greatly restricted. The plants in solution A, lacking iron, were growing satisfactorily, but the leaves were a lighter green than those of the plants in solution C, containing iron. The nutrient solutions were renewed, but the concentration of the pyrrole salt was reduced to one half the amount originally employed.

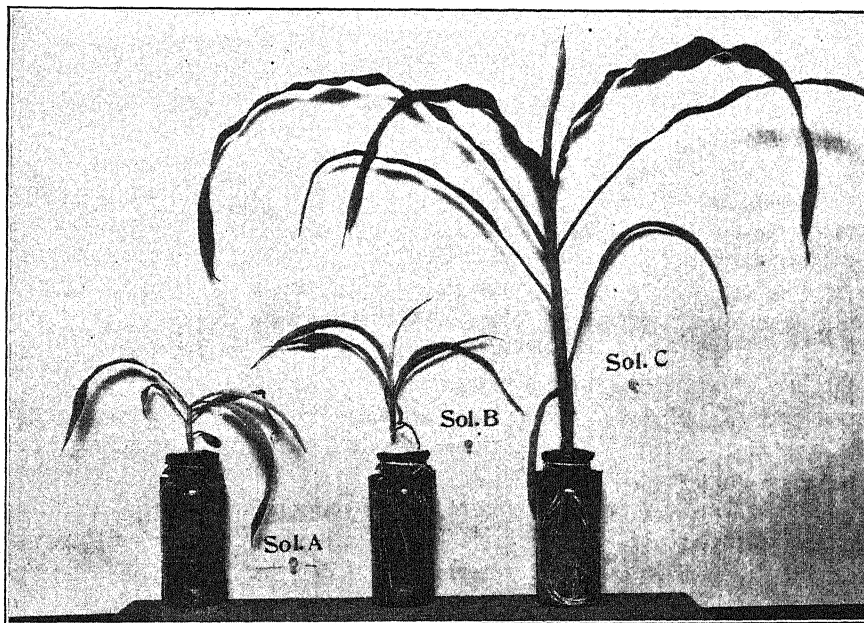
In the course of another week the roots of the plants in the solutions containing the pyrrole salt were injured to such an extent that the leaves rolled and wilted from lack of a proper water supply. This condition was overcome partially by placing the developing brace roots in the solutions. By the end of 7 weeks, only 3 of the plants receiving the pyrrole salt were still alive. These plants were very small, the new leaves were chlorotic, and the root systems were discolored. The development of lateral roots was particularly restricted. No difference was observed between the plants receiving phosphorus with the pyrrole salt and those without this element.

The plants in the solution minus iron were stunted in growth and the leaves were chlorotic, but the root systems were white and well branched. The plants supplied iron were 3 to 4 times the size of those lacking this element or supplied the pyrrole salt. The leaves of these plants were dark green and the root systems were in healthy condition. The appearance of representative plants at this time is shown in text figure 1.

The results of this experiment demonstrated that the pyrrole salt made in our laboratory was toxic to corn plants at concentrations of 0.125 and 0.250 g. per liter, both in solutions containing phosphorus and in those without this element. A reduction of the concentration of the pyrrole salt by one half did not allow the plants to recover from their injured condition. The leaves of the plants first acquired a bluish-green color accompanied by increased anthocyanin content in the culms and sheaths, but later the new leaves became chlorotic. The roots of the plants were most severely injured, in many cases to the extent of not being able to supply sufficient water to the leaves.

Possible explanations for the injurious effects obtained are: (1) that the pyrrole salt employed differed from that used by Oddo and Pollacci; (2) that toxic impurities were present; (3) that the concentration of the pyrrole salt was too high; (4) that the action of microorganisms upon this complex organic salt might produce injurious decomposition products; (5) that the plants used were too young. The method of preparation of the pyrrole

salt, its decomposition temperature, and the analysis cited indicate that the material used was the magnesium salt of pyrrole carbonic acid. The compound was purified in all cases in the manner described by Oddo and Pollacci, and in several of the later experiments it was purified twice. The other factors mentioned as being probable causes of the injurious effects were investigated.



TEXT FIG. 1. Reid's Yellow Dent corn grown 7 weeks in solution A, lacking iron, solution B containing 0.125 g. pyrrole salt per liter, and solution C containing 0.030 g. ferric chlorid per liter.

The concentration at which the pyrrole salt proved toxic in the experiment described above is that which Pollacci and Oddo used in their experiments. Nevertheless it was thought advisable to determine the effect of reducing the concentration.

#### EFFECT OF REDUCING THE CONCENTRATION OF THE PYRROLE SALT

Green and yellow seedlings of a strain of corn (5463-13  $\otimes$ ) kindly supplied by Dr. E. W. Lindstrom were grown with the pyrrole salt at several concentrations both with and without phosphorus. In solution B the pyrrole salt was used at rates of 0.025, 0.050, and 0.125 g. per liter, and in solution A at rates of 0.025, 0.050, 0.125, and 0.230 g. per liter. The check solutions A and C were also maintained, the latter containing 0.03 g. ferric chlorid per liter. Two 6-day-old seedlings, one green and one yellow, were placed in each culture.

In 10 days the green seedlings in all the solutions containing the pyrrole salt were slightly chlorotic, while those in solution *A* were distinctly chlorotic. The yellow seedlings showed no tendency to develop chlorophyll either in the solutions containing the pyrrole salt or in the solution with iron. When the solutions were renewed in 2 weeks, the root systems of all the plants in the solutions containing more than 0.025 g. of pyrrole salt per liter were discolored and the lateral roots were stubby and swollen at the tips.

The yellow seedlings in all the solutions grew very little after the first 2 weeks. The leaves of these plants became water-soaked, and within another 2 weeks all had died without becoming green.

When the experiment was concluded, in 4 weeks, the green plants in solutions containing 0.125 and 0.23 g. pyrrole salt per liter were stunted in growth, the leaves were chlorotic, and the roots were severely injured. With the lesser concentrations of this salt, 0.025 and 0.050 g. per liter, the growth of the plants was stunted and the leaves were chlorotic, but the roots were much less injured than in the solutions with the higher concentrations. In general, the plants in the solution lacking phosphorus, solution *B*, were in a slightly better condition than those in solution *A*, containing phosphorus and the pyrrole salt. The plants grown without iron were very chlorotic while those supplied with iron were large and normal in all respects.

The results of this experiment show that a reduction of the concentration of the pyrrole salt to 0.025 g. per liter decreased the injury to the corn plants but did not prevent the green plants from becoming chlorotic nor did it induce chlorophyll-development in the yellow plants. The growth of the green plants supplied with iron was greatly superior to the growth of those supplied with the pyrrole salt. The presence of phosphorus increased the injury caused by the pyrrole salt.

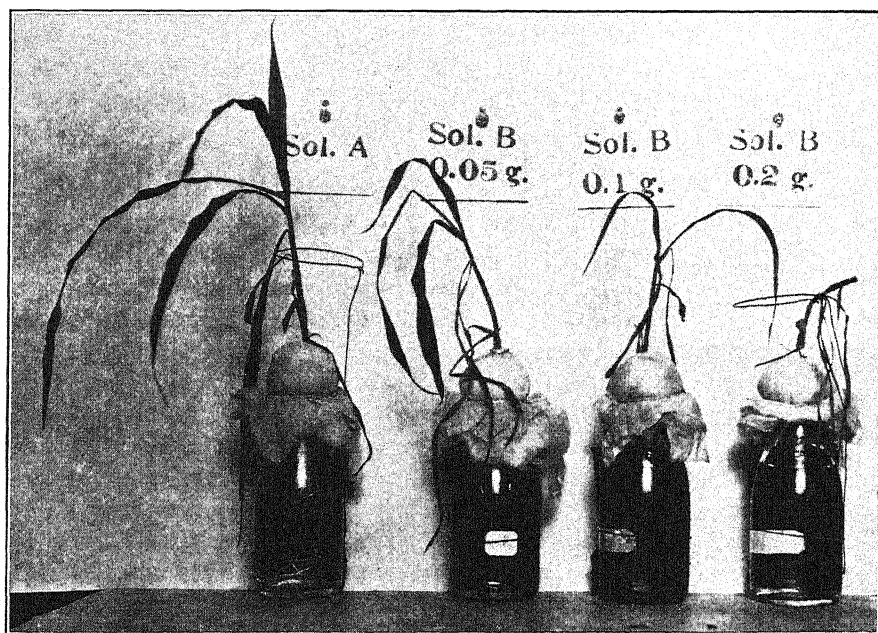
In the above-described experiment it was observed that the nutrient solutions containing the pyrrole salt became turbid. This suggested the presence of bacteria. A few drops of the turbid nutrient solution were transferred to a freshly prepared culture of solution *A* containing 0.125 g. pyrrole salt per liter. In 36 hours at 28 C. the solution became turbid, and microscopic examination showed the presence of rod-shaped bacteria. The possibility of bacterial activity producing toxic decomposition substances from the pyrrole salt was at once apparent. To eliminate the activity of bacteria, experiments were carried out under sterile conditions.

#### EXPERIMENTS WITH CORN PLANTS SUPPLIED THE PYRROLE SALT UNDER STERILE CONDITIONS

The methods of Wilson (7) for growing large plants in sterile media were used. Some of the nutrient solutions were sterilized by autoclaving at 18 lbs. pressure for 15 minutes, while others were sterilized in an Arnold

sterilizer for 30 minutes on 3 successive days to avoid the possibility of decomposing the complex pyrrole salt by the high temperatures of the autoclave. Longfellow flint corn was used in all the trials. Solution *B* containing 0.05 to 0.23 g. of pyrrole salt per liter, and solution *A*, lacking iron, were used in all these tests.

The results of every trial with the pyrrole salt in sterile solutions showed that it was as toxic under these conditions as under non-aseptic conditions. Text figure 2 illustrates the appearance of a typical series of corn plants grown under sterile conditions for 30 days.



TEXT FIG. 2. Longfellow flint corn grown 30 days with the roots under sterile conditions in solution *A*, lacking iron, and in solution *B* containing 0.05, 0.10, and 0.20 g. pyrrole salt per liter respectively.

Although Oddo and Pollacci used corn plants in their experiments with the pyrrole salt, it was thought that more favorable results might be secured with other plants since all the work with corn had given negative results. Experiments were therefore performed with cowpea, soybean, and *Spirodela*. At the same time that cowpea plants were grown in solutions containing the pyrrole salt, some of the plants were treated with a solution of this salt applied to the leaves as a paint.

### THE INFLUENCE OF THE PYRROLE SALT UPON COWPEA AND SOYBEAN PLANTS

New Era cowpea seedlings and Wilson soybean seedlings were grown in solution *B* containing 0.10 g. pyrrole salt per liter, in solution *A*, lacking iron, and in solution *C* containing 0.01 g. per liter of ferrous sulfate. Two cultures of solution *B* minus the pyrrole salt were set up so that this salt could be applied to the leaves.

When the experiment was concluded, after 21 days' growth, the toxicity of the pyrrole salt at a concentration of 0.10 g. per liter to both cowpea and soybean plants was evident. The most severe injury was sustained by the roots of the plants, while the leaves became chlorotic or died early. When the pyrrole salt in aqueous solution was applied to the leaves of cowpea plants the majority of the developing leaves were killed, a few became chlorotic, and one became a light mottled green. This method of supplying the pyrrole salt did not injure the root systems.

### THE USE OF RELATIVELY DILUTE CONCENTRATIONS OF THE PYRROLE SALT WITH COWPEA PLANTS

It was thought that beneficial effects of the pyrrole salt might be obtained in concentrations low enough to escape its toxic action. Six-day-old cowpea seedlings were therefore grown in solution *B* containing the pyrrole salt at rates of 0.001, 0.005, 0.010 and 0.020 g. per liter. The solutions were renewed every 3 days.

Within 10 days the leaves of the plants in all the solutions containing the pyrrole salt were yellow-green. A few plants formed several compound leaves, but they were small, malformed, and chlorotic with a slight tendency toward greenness about the main veins. The lateral roots of the plants receiving 0.010 and 0.020 g. pyrrole salt were very short and swollen at the tips. All the plants receiving the pyrrole salt died within 4 weeks. Reducing the concentration of the pyrrole salt reduced its toxicity but did not permit the development of chlorophyll in the absence of iron.

### GROWTH OF SPIRODELA POLYRHIZA WITH THE PYRROLE SALT

Plants of *Spirodela polyrhiza*, a floating aquatic, were used in one experiment to observe the influence of the pyrrole salt on mature plants since the previous work had been with seedlings. A concentration of 0.10 g. pyrrole salt per liter was used in solution *A* diluted 10 times and checked with solution *A* minus iron and solution *A* containing ferric chlorid at the rate of 0.010 g. per liter.

Injury to these plants was noticeable after 4 days, many of the leaves becoming yellow to brown in color. The roots developed dark-colored wellings near the tips, lacked the normal chlorophyll content, and soon became flaccid. The experiment was concluded after 25 days. Little

growth had been made by the plants in the pyrrole solution, while many had died and those still remaining were yellow and very small. The roots of the majority of the plants were decaying. The plants in the solution lacking iron had made fair growth but the leaves were chlorotic, while those supplied with iron made excellent growth and were normal in every respect.

#### EXPERIMENTS WITH ODDO'S PYRROLE SALT

Early in the present investigations, when it was found that the pyrrole salt used was injurious to corn plants, the writer communicated with Professor Oddo to see if he had experienced a similar difficulty or could explain the reason for the toxic action. He was of the opinion that toxic impurities were present in our compound and emphasized the necessity of protecting the pyrrole salt from light and renewing the nutrient solutions often. He also kindly supplied a sample of his compound.

Using this salt, an experiment was conducted with cowpea plants that had been grown 14 days in a nutrient solution lacking iron. The first compound leaves of these plants were about half developed and were only slightly lighter green than the primary simple leaves. The Oddo and Missouri pyrrole salts were used at concentrations of 0.20 g. per liter in solution *A* and checked with solution *A* minus iron, and with the same solution containing 0.005 g. iron as ferric chlorid. The solutions were renewed every 4 days.

In 4 days it was observed that the new leaves of the plants in the pyrrole salt solutions were slightly lighter green than those of the plants receiving iron. On the following day the primary leaves began to become yellow. By the seventh day the plants receiving pyrrole salt had lost their primary leaves, and the compound leaves remained small, were yellowish green, dying at the tips and around the margins, and several were completely wilted. These signs of injury were slightly more advanced in the plants growing in the solution containing the Missouri pyrrole salt.

The plants in the pyrrole salt solutions were so severely injured by the eighth day that the experiment was discontinued. In the solution containing Oddo's pyrrole salt 2 plants were completely defoliated, and one retained a compound leaf of light green color injured at the tips and around the margins of the leaflets. The roots of these plants were slightly discolored and flaccid. The condition of the plants in the solution containing the Missouri pyrrole salt was practically the same as described for those in the solution containing Oddo's compound. The only compound leaf remaining was very light green in color. The plants supplied with iron were in a normal growing condition, each having 2 or 3 well developed dark green compound leaves and turgid, white root systems. The plants lacking an iron supply had light green and white compound leaves but normal roots.

A very heavy growth of bacteria was observed in the solutions con-



taining the pyrrole salts, causing the solutions to be turbid and zoöglæa to form on the surface. Bacterial decomposition of the pyrrole salts may have markedly increased the injury to the plants. The solutions without the pyrrole salts were clear.

In a second experiment the Oddo and Missouri pyrrole salts were used at a concentration of 0.10 g. per liter in solution A with 6-day-old Long-fellow flint corn seedlings. The solution containing Oddo's compound was renewed on the fifth and ninth days. The supply of the Missouri compound was exhausted, so this solution was not changed.

During the first 5 days no differences were noticed between the plants supplied the pyrrole salts and those without it or supplied with iron. By the ninth day the tops of the plants supplied with the pyrrole salts appeared slightly smaller and a trifle more green than the check plants. The roots, however, were decidedly smaller than those of the check plants and were slightly discolored and flaccid.

A growth of molds developed about the corn grains, so the endosperms were removed. By the thirteenth day the leaves of the plants in the pyrrole solutions were badly wilted. An examination of the root systems showed those of the plants in the pyrrole solutions to be flaccid and about one half to two thirds the length of the check plants. Some of the lateral roots had swollen tips. The solutions containing the pyrrole salts were turbid, this condition indicating bacterial growth.

#### DISCUSSION

The results of the experiments performed by the writer fail to confirm the ability of a magnesium salt of pyrrole carbonic acid to replace iron in the development of chlorophyll by plants. They show, on the contrary, that the compound is quite toxic at concentrations considerably less than those reported by Oddo and Pollacci at which plants developed normally.

Why the results reported by the writer and those reported by Oddo and Pollacci should be so divergent can not be explained. All possibilities save those of different environments have apparently been eliminated. The experiments with the salt synthesized at the University of Missouri have taken into account the purification of the pyrrole salt, suitable nutrient solutions, the concentration of the pyrrole salt, the exclusion of light from the solutions, the renewal of the solutions, the age of the plant material, the action of microorganisms on the organic salt, and variation in kinds of plants. There still remained the possibility that the salt synthesized at the University of Missouri, although agreeing in empirical formula with the theoretical, might be an isomer of that used by Oddo and Pollacci. The limited experiments performed with the salt secured from Oddo show, however, that it acts upon plants under our conditions like the material prepared here.

## SUMMARY

1. Corn, cowpea, soybean, and Spirodela plants were grown in nutrient solutions containing a magnesium salt of pyrrole carbonic acid substituted for iron. In no case did this compound prevent chlorosis of the leaves of the plants. This result is contrary to those reported by Oddo and Pollacci.

2. In water cultures the pyrrole salt at concentrations of 0.001 to 0.250 g. per liter was toxic to the plants used.

3. The pyrrole salt was toxic to cowpea plants when applied to the leaves as a paint.

4. The decomposition point and analysis of the pyrrole salt used in this investigation indicated that it was the same salt as that used by Oddo and Pollacci.

5. Microorganisms develop luxuriantly in a nutrient solution containing the magnesium salt of pyrrole carbonic acid.

6. Under sterile conditions the pyrrole salt proved toxic to corn plants.

7. A sample of the pyrrole salt secured from Oddo was toxic to cowpea plants at a concentration of 0.20 g. per liter and to corn plants at a concentration of 0.10 g. per liter.

The writer wishes to express his sincere thanks to Dr. W. J. Robbins for many helpful suggestions and for his close interest in this investigation.

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# EFFECT OF SMUT ON SAP CONCENTRATION IN INFECTED CORN STALKS

ANNIE MAY HURD-KARRER

(Received for publication July 29, 1925)

It is probable that the material used in the development of the large galls of *Ustilago zae* (Beckm.) Ung. on corn (*Zea mays* L.) is derived from the solutes in the sap of the host plant. The question then arises as to whether the infected plant is able to replace these substances as they are removed by the growing gall, or whether the sap remains permanently impoverished. In order to obtain information on this question, comparative measurements were made of the specific gravity of the expressed juice of normal and of infected corn plants. These measurements, while showing the effect of the growth of the parasite on the total concentration of solutes in the sap, do not, of course, give any indication of the type of substances affected.

## METHODS

The plants for this study were obtained from the Arlington Experiment Farm through the courtesy of Mr. C. H. Kyle, agronomist in corn investigations. Only those plants were chosen for study which had actively growing galls on the stalks, and for which healthy control plants could be found in adjacent hills of the same row. For each comparison a control plant was selected which had the same number of nodes as the smutted plant and which resembled it closely in height, vigor, and stage of development.

After removal of the tough cortex, selected sections of the stalk were ground to a pulp from which the juice was squeezed out by hand through a muslin cloth. This juice was then filtered through a folded filter paper several times until clear. Precautions were taken to minimize evaporation throughout the experiments.

The specific gravity of the juice was determined by means of a small pycnometer in the form of a glass capsule with capillary intake and outlet tubes. A second weighing of the pycnometer filled with juice filtered again through the same paper constituted a check on each measurement. The two weighings practically always agreed within 0.0002 gram. The specific-gravity measurement was obtained from the weight of the sample of juice and the weight of an equal volume of distilled water at approximately the same temperature as the juice.

## RESULTS

The plants used for preliminary experiments in 1923 were 4.5 months old, and about 2.5 meters in height. In these experiments only those

internodes on which smut galls were growing, or which adjoined infected internodes, were selected from the diseased stalks for comparison with the corresponding internodes of the healthy control plants. Usually the juice from two or more such internodes was expressed as one sample, as indicated in the tables.

The results recorded in table 1 indicate that infection of corn by smut results in a lower specific gravity of the juice in the infected stalks.

TABLE 1. *Comparative Determinations of the Specific Gravity of Juice of Smutted and of Healthy Corn Stalks about 18 Weeks Old*

Date (1923)	Internodes (Numbered from Base of Stalk)	Specific Gravity of Juice	
		Smutted Plants	Healthy Plants
Sept. 29. ....	2 and 3	1.0237	1.0298
	5 and 6	1.0310	1.0354
	10, 11, 12, and 13	1.0363	1.0420
Oct. 1. ....	5 and 6	1.0311	1.0452
Oct. 1. ....	7 and 8	1.0365	1.0520
Oct. 2. ....	6 and 7	1.0489	1.0556
Oct. 3. ....	2	1.0151	1.0319
	5 and 6	1.0160	1.0401
Oct. 3. ....	2 and 3	1.0105	1.0190
	6 and 7	1.0199	1.0361

TABLE 2. *Comparative Determinations of the Specific Gravity of Juice of Smutted and of Healthy Corn Stalks, 16 to 19 Weeks Old*

Date (1924)	Internodes (Numbered from Base of Stalk)	Specific Gravity of Juice	
		Smutted Plants	Healthy Plants
Sept. 16. ....	2 and 3	1.0382	1.0415
	4	1.0386	1.0441
Sept. 20. ....	4 and 5	1.0449	1.0485
Sept. 20. ....	4, 5, and 6	1.0459	1.0468
Oct. 1. ....	3	1.0346	1.0371
	5	1.0371	1.0394
	7	1.0416	1.0439
	8	1.0421	1.0457
Oct. 2. ....	7	1.0358	1.0481
	10 and 11	1.0410	1.0496
Oct. 2. ....	9	1.0313	1.0407
	10 and 11	1.0330	1.0422
Oct. 5. ....	5	1.0280	1.0340
	8 and 9	1.0328	1.0441
Oct. 5. ....	5	1.0384	1.0469
	7 and 8	1.0451	1.0515



In table 3 are given measurements on juice from four diseased stalks with the corresponding values for the healthy control plants in parallel columns. The plants were 18 weeks old, with ears in the soft-dough stage. In table 4 are recorded measurements on four more diseased stalks obtained during the week following, when the ears were in the hard-dough stage. The positions of the galls on the smutted stalks are indicated by asterisks.

TABLE 4. *Comparative Determinations of the Specific Gravity of Juice of Smutted and of Healthy Corn Stalks about 18 Weeks Old*

Internode (Num- bered from Base of Stalk)	Oct. 2, '24		Oct. 3, '24		Oct. 3, '24		Oct. 4, '24	
	Diseased Plant	Healthy Plant	Diseased Plant	Healthy Plant	Diseased Plant	Healthy Plant	Diseased Plant	Healthy Plant
1.....	1.0243	1.0346	1.0133	1.0382	1.0262	1.0387	1.0264	1.0301
3.....	1.0240	1.0376	1.0127	1.0409	1.0243	1.0378	1.0261	1.0295
5.....	1.0259	1.0382	1.0147	1.0415	1.0268	1.0414	1.0282	1.0339
6.....	.....	.....	*1.0172	1.0445	1.0270	1.0418	.....	.....
7.....	1.0255	1.0395	*1.0184	1.0458	*.....	.....	1.0303	1.0372
8.....	*1.0259	1.0398	*1.0202	1.0469	1.0274	1.0449	*1.0315	1.0381
9.....	*1.0268	1.0404	.....	.....	*.....	.....	*1.0337	1.0396
10.....	} 1.0285	1.0413	} 1.0267	1.0512	} 1.0295	1.0461	.....	.....
11.....							.....	.....
12.....							.....	.....
13.....							.....	.....
14.....	} 1.0309	1.0427	} 1.0346	1.0554	} 1.0345	1.0473	1.0369	1.0400
15.....							.....	.....
16.....							.....	.....
17.....							.....	.....

The results reported in tables 3 and 4 confirm the conclusion that one of the effects of infection of corn by smut is a lowering of the sap concentration. It is also evident that this effect often is not limited to those internodes of the stalk in the immediate vicinity of the galls. In many plants with but two or three galls near the middle of the stalk, the specific gravity of the juice was lower throughout the entire stalk than in corresponding internodes of the healthy control plants. The most striking instances of this condition are recorded in table 4.

Although the growth of the parasite often lowered the sap concentration throughout the stalk, the effect was most pronounced in internodes adjacent to galls. In fact, the juice from sections adjoining actively growing galls was often so impoverished that the normal concentration gradient was actually reversed. Such a reversal of the gradient was never found in a healthy stalk except in the basal internodes.

The lower concentration of solutes in the juice of diseased stalks may have been due to the inability of the infected plants to elaborate or to transport food materials at a normal rate, as well as to the removal of such substances by the parasite. But, since there was no appreciable leaf infection, and since the vigor and normal development of the infected plants were in no

case visibly affected, it seems probable that the reduction in sap concentration was due to utilization of the soluble carbohydrates by the fungus.

#### CONCLUSIONS

1. The specific gravity of juice expressed from smut-infected corn stalks bearing actively growing galls is lower than that of juice expressed from the corresponding internodes of healthy control plants.

2. This lowering of the sap concentration, although most pronounced in the internodes adjoining a smut gall, often is evident throughout the stalk.

3. The specific gravity of the sap is sometimes so low in the internodes adjoining the attachment of galls as to reverse the concentration gradient in that section of the stalk. Such a condition was never found in healthy stalks, nor in diseased stalks except in the immediate vicinity of actively growing galls.

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## A BASIS FOR AGREEMENT ON NOMENCLATURE AT THE ITHACA CONGRESS

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There is an insistent demand among botanists, especially among the non-taxonomists, that plant nomenclature be unified and stabilized—that the taxonomists “get together” on nomenclature. There is an impression that the present unsettled condition in nomenclature is a recent development due to conflicting codes, that if we could get back to the good old names all would be well. On the contrary there has never been a period since plants were given technical names when there were not complaints and criticisms concerning the “changing of well known names.” Beauvois more than one hundred years ago found it necessary to explain his failure to conserve “certain names already in use” and argues:

If botanists will adopt this principle [use of the oldest name, beginning with the works of Linnaeus] there will be in the future neither arbitrariness nor confusion in nomenclature . . . Mibora will no longer be called *Knappia* in England and part of Germany; it will not be *Sturmia* for one, *Chamagrostis* for the other; it will be *Mibora* throughout the world and botanists will understand one another much better.

Does this not have a familiar ring? Codes of nomenclature have been adopted in an attempt to bring order out of confusion and not, as some seem to think, to introduce more confusion.

The non-taxonomist wishes to have one name for one plant, constant, invariable, and everlasting throughout the world. Those who were raised on the fifth edition of Gray's Manual, as was the writer, lamented the changes of well known names that came out in succeeding editions. We remember names as we learned them. To each, whether raised on “Gray,” “Wood,” or “Britton and Brown,” the well known name is the one familiar to *him*.

There is also an impression that the changes in names are due to the excessive activity of name-jugglers who are constantly searching for early names, bringing them to light, polishing them up, and setting them in place of the names we know, and placing their own names after the new combinations. There may be some slight basis for such an impression since occasionally triflers invade taxonomy as they may other branches of botany, but their influence, on the whole, has been small.

Changes of names are due to (1) differences in taxonomic opinion,

<sup>1</sup> Published, at the expense of the author, out of the order determined by the date of receipt of the manuscript.



(2) synonyms and homonyms, (3) incorrect identification. Let us examine these categories.

#### 1. CHANGES OF NAMES DUE TO DIFFERENCES IN TAXONOMIC OPINION

"A name is an expression of a taxonomic idea." The limits of genera and species are matters of taxonomic opinion. This opinion is based upon knowledge of the morphology of the group in question but is influenced by the student's attitude toward the relationships of groups. Some are by nature "lumpers," others are "splitters," but all conscientious work takes into consideration the known facts. The arrangement or limitation of taxonomic groups is an attempt to express genetic relationships. It is clear that new facts may alter the opinion concerning relationships, and this changed concept may be reflected in the names by which the revised taxonomic ideas are expressed. Taxonomy is based chiefly on morphology. Morphology is not yet a completed study. The discovery of new facts is likely to continue for an indefinite period. So long as morphology is a living subject, so long may we expect changes in taxonomic concepts, thus necessitating changes in names. A large percentage of the changes of names comes in this category.

#### 2. CHANGES OF NAMES DUE TO SYNONYMS AND HOMONYMS

Two or more names for a single taxonomic concept (synonyms) or the same name applied to two or more taxonomic concepts (homonyms) are the chief causes of confusion in nomenclature, and it is with this source of confusion that codes are chiefly concerned. Of these synonyms and homonyms some have been accepted in one part of the world and not in another, or by certain botanists and not by others. Some names, perchance the earliest, may have received no recognition by subsequent authors. Personal loyalties or national pride may have entered into the situation. Furthermore, when a species is transferred from one genus to another the species name may be already in use in the second genus, so that further change is required.

Botanists have found it necessary to establish rules (codes) to govern procedure when changes are made. No code can prevent changes in names. As has been shown above, changes are necessary and inevitable so long as taxonomy and morphology are living sciences. But a code should show how changes are to be made so that botanists may all make them in the same way.

In order to be of permanent value as a guide, a code must be founded on principles recognized and accepted by botanists in general. The details can be formulated only approximately because botanists can not foresee all possible cases nor the effect of the provisions in every case. There should be an opportunity for a revision of a code at intervals as dictated by experience. Botanists of the present can not bind those of the future. They may be able to agree on rules that compromise divergent views, but

unless these compromises appeal to future botanists as reasonable they will not be followed.

### 3. CHANGES OF NAMES DUE TO MISAPPLICATION OR INCORRECT IDENTIFICATION

Under categories 1 and 2 we have considered names as published by their authors. It is with such names that codes are concerned. But a serious source of confusion in the use of names is the misapplication of names by subsequent authors. It is easy to understand how such erroneous use took place; it is exceedingly difficult to clear up the confusion caused by it. Most early descriptions were very brief and inadequate, and there was little knowledge of plant geography. An author in writing a flora of a region may have misidentified some of his species, applying names to them that were originally applied to other species in another region. When such a work becomes the accepted manual or flora of a region these misapplied names have, to those who use the manual, all the "authority" of original publication.

For example, in the fifth edition of Gray's Manual a certain grass appears under the name *Panicum pauciflorum* Ell. Later it was thought that the grass there described was not the same as the closely related southern plant to which Elliott first gave the name. Hence in the sixth edition of the Manual *Panicum pauciflorum* was eliminated.

The discarding of *P. pauciflorum* was due to taxonomic opinion. Unfortunately, however, the name which was substituted was *P. scoparium* Lam., a species so different that no botanist had considered it even closely allied to *P. pauciflorum*. Here was a change based not on taxonomic opinion but on an erroneous identification. In the seventh edition of the Manual the error was corrected. *Panicum scoparium* Lam. was applied to the proper grass and the correct name, *P. scribnerianum* Nash, given to what had been called *P. scoparium* in the sixth edition. There was much regret and some irritation among users of the manual because of the change of this and other well known names, but a moment's consideration will show that if anyone is culpable it is the one who misapplied the name, not the one who, after studying the case anew, corrects it.

Changes in this category are necessary and inevitable until botanists cease to make mistakes. In extenuation of such errors it should be added that botanists have sometimes been forced to work with scant material or with insufficient library facilities, or have not been able to refer to the original specimens or types.

### CODES OF NOMENCLATURE

The first serious attempt at international agreement was made at Paris in 1867 and resulted in the Paris Code. Experience showed that, as is not unusual when laws are proposed, the makers of the code had not

foreseen all cases nor the effect of some of the provisions. In 1905 at Vienna another attempt was made to formulate a code. This, the Vienna Code, was based upon the Paris Code but the provisions were greatly extended and modified.

About this time a group of American botanists formulated a set of rules known as the American Code. This was presented for consideration at Vienna but was not accepted. The American differed from the Vienna Code in two fundamental respects. It introduced the concept of types in determining the application of names, and it attempted to apply the principle of priority uniformly and rigidly.

Since 1905 American botanists have been divided in their support of these codes. A canvass of the Botanical Society of America a few years ago showed that approximately half of the taxonomists supported each code. There have been attempts to bring about an agreement on the basis of a compromise between the two codes or by a modification of the International Rules (Vienna Code), but some of the supporters of each have resisted this. The rigid supporters of the Vienna Code maintain that it is international and represents the majority opinion of the botanists of the world, while those of the American Code maintain that it is founded upon fundamental principles of inherent correctness and must ultimately prevail.

#### THE CODE OF THE FUTURE

In the writer's opinion the future code will be a modification of the International Rules which will include certain important features of the American Code. Such modification is even now under way. At the Brussels Congress (1910) the type concept of the American Code was recognized to the extent that a recommendation was added to the International Rules (Vienna Code) that types of species and genera be indicated in the future.

The Type-basis Code formulated by the Committee on Nomenclature of the Botanical Society of America (1919) modified the American Code by making it more flexible and introduced the idea of exceptions to the rules which should be validated by a judicial body.

Recently the British botanists proposed certain modifications of the International Rules at the Imperial Conference. The suggested changes would bring the rules in accord with the Type-basis Code on nearly all important points.

At the coming congress at Ithaca (International Congress of Plant Sciences, August 16-23, 1926), the British botanists will present these proposals for discussion, and the Botanical Society of America has passed a resolution providing for the examination of these proposals at that time.

In view of the discussion which will take place at this congress, the writer wishes to bring to the attention of botanists the important differences between the two codes and the probable direction which mutual modification

may take. Continued discussion should be helpful toward a final agreement, especially if it can be carried on impersonally and without prejudice or controversy. All botanists desire that there should be an agreement on nomenclature, but the details of this agreement must be worked out by taxonomists.

At this point it may be well to refer to the recommendations of the Imperial Botanical Conference held at London in 1924.

#### IMPERIAL BOTANICAL CONFERENCE

The conference adopted a set of resolutions prepared by its committee on nomenclature (Dr. A. B. Rendle, chairman).<sup>2</sup> These resolutions are to be presented to the International Congress at Ithaca.<sup>3</sup> It would be well for all those interested in nomenclature to read the report of this committee (p. 301). The discussion was led by Mr. T. A. Sprague and was participated in by many botanists. The report was finally adopted unanimously by the conference. The arguments in favor of the adoption of the type concept could not have been more convincing if they had been set forth by an advocate of the Type-basis Code. The report favors the term "standard" in place of "type" (standard species, standard specimen). In cases in which it is desirable to conserve genera against the action of the rules a standard species should be indicated, which, however, may not be identical with the type species.

The Conference finally adopted the resolution, "The principle of the type-method of applying names should be formally accepted."

Other resolutions adopted were the following which bear especially upon a compromise between the two codes:

That the rule requiring Latin diagnoses after 1908 should be replaced by a recommendation to supply Latin diagnoses in the future.

That all combinations which are later homonyms should be rejected.

That all generic names which are later homonyms should be rejected except such as may be specially conserved.

That duplicate binomials should not be rejected.

That the list of conserved names should be revised.

Nearly all these modifications would act in the direction of the Type-basis Code. The word *compromise* has been used above by the writer. However, the proposals of the British botanists to modify the International Rules and the suggestions of the writer to modify the Type-basis Code are not made primarily as a compromise, but rather to incorporate elements which experience has shown will make the codes more workable. As a result of such changes they would approach agreement.

<sup>2</sup> Imperial Botanical Conference. Report of Proceedings. Cambridge, 1925.

<sup>3</sup> The International Congress of Plant Sciences (Fourth International Botanical Congress), Ithaca, N. Y., August 16-23, 1926. Organization Committee, B. M. Duggar (chairman), H. C. Cowles, H. H. Whetzel. Chairman, Committee on Taxonomy, K. M. Wiegand. See also Amer. Jour. Bot., Jan., 1926, page 4 of cover.

## DIFFERENCES BETWEEN THE INTERNATIONAL RULES AND THE TYPE-BASIS CODE

In a preceding paper the writer pointed out the important differences between the two codes.<sup>4</sup> They differ chiefly in 8 respects:

(1) The type concept, (2) starting-point for nomenclature, (3) exceptions to the priority rule, (4) publication of genera, (5) priority of position, (6) validity of homonyms, (7) duplicate binomials, (8) Latin diagnosis.

### I. TYPE CONCEPT

This is not included in the International Rules but is not contrary to their spirit. At the Brussels Congress a recommendation was added to the rules to the effect that in the future authors should indicate the types of new genera and species. The type concept is one of the fundamental principles of the Type-basis Code. The advantages of the type concept are becoming generally recognized by the advocates of the International Rules, and it is probable that the rules will be modified to include this concept. It is also probable that the modification will include the idea of standard species for those cases in which the type species would lead in a direction contrary to usage. The Botanical Society of America at its last meeting recommended that the International Rules be modified to include a recommendation that the authors of revisions of taxonomic groups indicate what they accept as the type species of genera and the type specimens of species. We may assume that this difference between the codes will soon disappear.

### 2. STARTING-POINT FOR NOMENCLATURE

Both codes take 1753 as the starting-point for the flowering plants. The International Rules accept different dates for starting-points for different groups of Cryptogams (Brussels Congress). The botanists concerned with the lower groups must ultimately come to an agreement on this, but with the modifications proposed by the British they may find that 1753 will be satisfactory for all groups.

### 3. EXCEPTIONS TO PRIORITY RULE

This is the most important difference between the codes, and probably both codes must be modified to reach an agreement. Many followers of the Type-basis Code admit that some genus names in general use should be conserved. If the supporters of the International Rules are willing to follow the suggestion of the British botanists that the list of conserved names be revised, it seems probable that an agreement can be reached on this point. The list appended to the International Rules evidently was never revised to accord with the rules as finally adopted by the congress. It contains many names that are not exceptions to the rules and thus unnecessarily extend the list. *Buchloe* and *Leersia* are conserved against

<sup>4</sup> Jour. Bot. Brit. For. 60: 316. 1922.

Bulbilis and Homalocenchrus, but the latter two would be invalidated by the rules themselves.

In the writer's opinion there should be a list of *nomina conservanda*, but the present list should be revised. An agreement on a list of conserved names can be more readily reached if it is accepted that the rules shall prevail except when their strict application would result in changing well known and long-accepted names in two categories: first, genera containing a large number of species; and second, genera containing well known economic species.

The revision should be done by a competent international committee of taxonomists, who shall decide the many difficulties that will arise, including the interpretation of usage as applied to long-established names.

#### 4. PUBLICATION OF GENERA

The original American Code admitted effective publication of a genus if it could be definitely connected with at least one species, because thus the type could be determined. The International Rules require that there should be a generic description or a reference to a previously published genus name to constitute effective publication.

The Type-basis Code requires for effective publication that the genus name shall be accompanied by a binomial species name (Art. 2) or by a definite reference to such. This provision eliminates those names which had been accepted by the American Code on the basis of being "associable by citation with a previously published binomial species" (Canon 10), for example, Homalocenchrus Mieg.

An examination of the grass genera has been made to bring them in accord with this provision of the Type-basis Code. The following American genera are included in the list of *nomina conservanda* of the International Rules. In the right-hand column are placed the American Code names. The italicized names in the left-hand column are accepted under the Type-basis Code because the corresponding American Code names are not effectively published:

International Rules		American Code	
1. Rottboellia L. f.....	1779	Manisuris L.....	1771
2. Tragus Hall.....	1768	Nazia Adans.....	1763
3. Zoysia Willd.....	1801	Osterdamia Neck.....	1790
4. Leersia Swartz.....	1788	Homalocenchrus Mieg.....	1760
5. Hierochloa R. Br.....	1810	Torresia Ruiz & Pav.....	1798
6. Cynodon L. C. Rich.....	1805	Capriola Adans.....	1763
7. Ctenium Panz.....	1813	Campulosus Desv.....	1810
8. Buchloe Engelm.....	1859	Bulbilis Raf.....	1819
9. Diarrhena Beauv.....	1812	Diarina Raf.....	1808
10. Zeugites Schreb.....	1791	Senites Adans.....	1763
11. Lamarkia Moench.....	1794	Achyrodes Boehm.....	1760
12. Glyceria R. Br.....	1810	Panicularia Fabr.....	1763

It will be noted that six names out of the twelve are removed from the right-hand column, that is, the differences in usage of genus names based

on the list of *nomina conservanda* are reduced by 50 percent by the provisions of the Type-basis Code. If this proportion is found to prevail throughout the flowering plants, there will be a radical reduction in the number of names to be considered in a final adjustment. Even then the list must be open for additions as the consensus of opinion shall approve. If the advocates of the Type-basis Code are willing to concede the requirement of a generic description to establish effective publication, a still further reduction of differences is brought about. By agreeing so far as possible to exclude names which have been casually mentioned or published, but scarcely effectively so, the number of names remaining for actual consideration on the basis of priority *versus* usage (conserved names) would be relatively small.

#### 5. PRIORITY OF POSITION

In order definitely to establish validity of publication when two or more competing names were published on the same date, the American Code provided that the name having precedence of position should have priority. This is often referred to as priority of position. The International Rules leave this to the choice of the next author and his decision is to be followed.

The advocates of the Type-basis Code will probably concede this point, because it seems unreasonable to displace a well known name merely on the ground of precedence of position.

#### 6. VALIDITY OF HOMONYMS

The International Rules are vague on this point. They provide that "No one is authorized to reject, change, or modify a name (or combination of names) because . . . of the existence of an earlier homonym which is universally regarded as non-valid . . ." (Art. 50). This rule is difficult to apply. The British botanists say (Proc. Imper. Conf. 303): "As it is frequently a matter of opinion whether the first use of a name is a synonym or not, the conditional acceptance of homonyms leads to instability and uncertainty." Hence they propose, in their recommendations for the modification of the International Rules, that (a) all combinations which are later homonyms should be rejected, and (b) that generic names which are later homonyms should be rejected unless they are specially conserved. This seems entirely reasonable and probably will ultimately be accepted by botanists. The Type-basis Code rejects all later homonyms but provides for exceptions in special cases (Art. 7).

#### 7. DUPLICATE BINOMIALS

The International Rules reject duplicate binomials (such as *Phragmites phragmites*). The Type-basis Code accepts these. There is a tendency among the followers of the latter code to reject them, and the British

botanists are proposing to accept them. This difference in the codes is a minor point and should be readily adjusted.

#### 8. LATIN DIAGNOSIS

The International Rules provide (Art. 36) that "On and after January 1, 1908, the publication of names of new groups will be valid only when accompanied by a Latin diagnosis." There is no such provision in the Type-basis Code. The report of the British botanists, previously referred to, states that Article 36 does not comply with the general condition that "Rules should be reasonable, otherwise they will not be accepted; they can not be enforced," and was not in accordance with one of the leading principles on which the rules were based [Art. 3. The rules of nomenclature should neither be arbitrary nor imposed by authority, etc.]. The report considers the rule to be arbitrary and imposed by authority, and not founded on considerations forcible enough to secure general acceptance. The report proposes to revoke the rule and replace it by a recommendation to the same effect. The conference report states that 10,000 new species of flowering plants have been described without Latin diagnoses between 1908 and 1924.

Botanists generally recognize that Latin diagnoses are desirable, but many are not ready to invalidate names not accompanied by Latin diagnoses if otherwise properly published. A recommendation will probably be as effective as a rule and will not appear arbitrary.

#### SUMMARY

The program of the Section on Taxonomy of the Ithaca Congress (International Congress of Plant Sciences, August 16-23, 1926) will provide time for a discussion of nomenclature. The British botanists will present certain proposals for discussions. The Botanical Society of America has voted to examine these proposals, and to present certain modifications of the International Rules. The preceding synopsis of the present status of nomenclature is presented with the hope that it may direct the attention of the members of the Botanical Society to the items that are likely to be discussed.

Botanists look with regret or even irritation on changes in plant names, especially in those that are well known and long-established. Changes due to differences of taxonomic opinion must continue so long as morphology and taxonomy are living sciences. Changes due to synonyms and homonyms may be regulated by codes. Changes due to the misapplication of names ought to be made. Changes in the last two categories will become fewer as taxonomic study progresses.

A brief outline is given of the Paris Code, the Vienna Code, the American Code, and the Type-basis Code.

An outline of the British proposals is given.



The important differences between the International Rules and the Type-basis Code are outlined.

It is shown that in the American grass genera in the present list of *nomina conservanda* there is a reduction by 50 percent in the differences by applying the provisions of the Type-basis Code.

The list of *nomina conservanda* contains many names that are valid under the rules themselves and hence were not in need of conservation. The list should be revised for this and other reasons.

Agreement on a code can not prevent changes of names but it will provide an orderly method for making such changes as are necessary.

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# THE STRUCTURE OF THE CHLOROPLAST IN CERTAIN HIGHER PLANTS<sup>1</sup>

## PART I

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### HISTORICAL SUMMARY

Comparetti<sup>2</sup> (8; cit. Trecul, 88) seems to have been the first to mention the green granules which we now call chloroplasts. It appears that he also saw the grains of starch enveloped by the green material, though he never guessed their nature. Sprengel (82) and Treviranus (89; cit. Gris, 13), and later Turpin (92), believed that these green granules were really vesicles which he thought gave birth to new cells. Wahlenberg (95) held the chlorophyll in the living plant to be a viscous liquid which coagulated into globules only after it had been extracted. Link (29) spoke of the green pigment in the plant as "colored resinous material" and believed that it is present in the plant sometimes as a thick granular mass, sometimes as vesicles, and sometimes even as a thick lining at the periphery of the cell. Moldenhauer (cit. Gris, 13) believed that the grains of chlorophyll result from the coagulation of the green sap of the cells. Treviranus (90), eight years after his above-mentioned work, very nearly approximated our present view of the structure of the chloroplast when he stated that it consists of an albuminous globule with which the green material is mixed. Dutrochet (cit. Gris, 13) noted a great number of small green grains in the cells at the base of the petiole and considered them to be equivalent to the nerve corpuscles in animals. Mulder (51) stated that a grain of chlorophyll could bring about entirely "the metamorphosis of a grain of starch with the sole help of the azotic material dissolved in the cell." Meyen (cit. Gris, 13), though he later changed his views (38), admitted the vesicular nature of the chloroplast. Treviranus (cit. Trecul, 88) announced that these grains of chlorophyll swim in a green sap less dark than themselves, and that they result from a transformation of this sap, which is normally applied to the internal surface of the cell wall. Link also stated that a "green sap" is found throughout the cells of young stems and leaves exposed to light and that it is only rarely that this sap is localized in the vesicles. He held that it usually surrounds the vesicles (chloroplasts) somewhat as a cloud. He further noted that sometimes the vesicles are composite, that

<sup>1</sup> Botanical contribution from the Johns Hopkins University no. 82.

<sup>2</sup> References are to literature listed at the end of the second paper of this series, to appear in the following issue of the JOURNAL.

is, the larger vesicles contain smaller ones. Von Mohl (49), recognized that chlorophyll sometimes occurs in irregular masses, which he called "amorphous chlorophyll," and at other times in definitely formed grains, which later, when well developed, always show one or more starch grains surrounded by a green jelly.

Nägeli (53) protested against the view of von Mohl and most of his successors that the chlorophyll grains are composed of a nucleus of one or more starch grains on which is precipitated a coating of chlorophyll, since no one had seen this process occurring. If this view were correct, he urged, the starch grains must exist first and later be covered with chlorophyll. Moreover, in the algae, many chloroplasts were seen which contained no trace of starch. Nägeli held that the chlorophyll grain is a vesicle and should be classified with the other colored globules in the cell sap. He believed also that it is surrounded by a cellulose membrane, for when many lay close together they did not fuse into a mass, but became as cells "parenchymatisch." Indeed Nägeli assumed these vesicles (green and colorless) to be analogous to cells, and held that the cell is built up of these vesicles just as the organism is built up of cells.

Quekett (60), studying thin sections of the potato tuber, reported that he found starch developing into chlorophyll. Goeppert and Cohn (12) observed that if the chlorophyll grains were extruded in water they swelled; the green color became thinly spread on the surfaces of the globules while the remainder became colorless like water; the whole finally disappeared by dissolving. These observations lent great weight to the idea that the globules of chlorophyll are delicate cellular vesicles, composed of a transparent membrane which swells in water and which encloses a green fluid with many solid particles. Hofmeister (19) said:

They [the chloroplasts] are sometimes without doubt vesicles on the interior wall of which is applied the half-soft, transparent or clotted green substance; at other times they appear as small homogeneous masses which sometimes enclose solids. It is probable that even this latter kind of chlorophyll corpuscle is in reality vesicular when mature. . . . Then the colored particles develop further by uniting in spherical globules; these may later clothe themselves in a membrane and propagate by division.

Nägeli (54) modified somewhat his former view. He still held that the chlorophyll grains are vesicles with the green color restricted to the surface, but said that they are surrounded by cellulose membranes. He now considered them to be composed of a protein substance whose surface is covered by a condensation membrane formed by the contact of the grain itself with the cell sap. This membrane became especially distinct when the chlorophyll grains were placed in water, which, as in the case of chlorophyll of *Clivia*, etc., finally caused them to burst. The halves of the burst green shell or film had the appearance of two valves.

Von Mohl (50) also altered his original conception. He now recognized that, although starch and chlorophyll are frequently found together, they

nevertheless arise independently. He still held that the green "autoplasts" are membraneless in the state of nature, and that the membrane seen by Nägeli was an artifact due to the alteration of the chloroplast in water. Unger (93) stated that chlorophyll exists in an amorphous state, united in fillets or in globules. These latter, which often include starch grains, are surrounded by a vesicular membrane. Gris (13) held that the green basic mass is primary and the starch secondary. However, he noted that in some cases the formation of the grains of chlorophyll followed "the appearance of large kernels of starch which enveloped themselves in a green jelly and became isolated little by little." He also discovered that the chlorophyll bodies of seedlings would not turn green in the absence of iron.

Sachs (65-69) found that chlorophyll is always united to definite portions of the protoplast. The amount of pigment is relatively very small, for its removal affects neither the shape nor the volume of the ground substance, which is always a solid, soft body containing extremely small vacuoles in which the chlorophyll is generally, though not always, uniformly distributed. The chloroplasts are always imbedded in the cytoplasm and are never in contact with the vacuole or cell wall. With few exceptions, starch grains arise in the homogeneous solid substance of the chloroplasts. Minute at first, they increase in size and sometimes so completely fill the space of the chloroplast that the green substance appears as a thin coat about the mature starch grain; even this coat may disappear and the starch contents lie free in the cytoplasm of the cell in the place of the chloroplast. Sometimes drops of oil form in the interior of the chloroplast, and other granules of an unknown nature were observed by Sachs. At no time did he find evidence that a chlorophyll body could be derived in whole or in part from a grain of starch.

Hofmeister (20) further developed the views he had previously published. He now held that the chlorophyll grain is composed of two layers, the peripheral layer being noticeably denser than the inner one though passing over into it gradually. He frequently noticed that, when vacuoles or inclusions of any sort were present, the substance of the chlorophyll body next to such an inclusion seemed denser. This appearance was more readily seen in intense light, when the green coloring matter seemed to be somewhat more concentrated. The ground substance had a capacity for swelling in water. The inner mass of the chlorophyll body had a greater power of absorption than the peripheral layer. In general, when water was absorbed vacuoles appeared in the central mass. These vacuoles had a tendency to swell and fuse and finally burst through the peripheral layer.

Briosi (5) was the first to recognize oil droplets in the chloroplasts of a number of plants as perfectly normal inclusions and not the results of a pathological condition. He also showed that in several species of *Strelitzia* and *Musa* no starch occurs; but that oil droplets instead of starch grains arise in the chloroplasts of these plants and that this oil exists in finely divided particles between the "protoplasmic molecules" of the chloroplasts.

Haberlandt (14) found little change in the structure of the chloroplasts when they were reduced to a temperature of from  $0^{\circ}$  to  $-2^{\circ}$  C. When, however, they were subjected to a temperature of from  $-4^{\circ}$  to  $-6^{\circ}$  C. they were notably altered; while at a temperature of from  $-12^{\circ}$  to  $-15^{\circ}$  C. they were completely destroyed. The chlorophyll grains of evergreens, on the other hand, became vacuolated in consequence of the cold and agglomerated into a larger clump.

Mikosch (47) held that the grains of starch could transform themselves into chlorophyll granules. And Stohr (85) also noted that the formation of the grains of chlorophyll was often preceded by the formation of grains of starch. Frommann (11) found in the functioning chloroplasts of phanerogams a very fine fibrillar structure. The whole plastid he held to be composed of a fundamental network of green threads. Schimper (72) sought to prove that a grain of starch is always produced in an "albuminoid corpuscle," *i.e.*, in a plastid which was itself produced by the division of a preëxisting plastid. He even attributed to these alone the rôle of starch-formation. He stated that a grain of starch is never produced directly from undifferentiated cytoplasm. Pfeffer (56) showed that the plastid can not carry on photosynthesis in the absence of the chlorophyll it normally contains. Pringsheim (58, 59) found that placing chloroplasts in water at a temperature of  $50^{\circ}$  to  $60^{\circ}$  for from five minutes to an hour, or in live steam, caused them to swell, to develop a large central vacuole, and finally to burst to fragments that often appeared as green hulls. After heating, a coloring matter which he called hypochlorin often exuded from the chloroplast in the form of small droplets, which remained in intimate contact with the ground substance. From a microscopic examination of the chlorophyll bodies in this condition, he came to the conclusion that the ground substance is a hollow sphere possessing a spongy structure, and that it holds the green coloring matter in its meshes. This green pigment he thought is only mechanically associated with the framework. The colored plates of his first paper still repay study.

Schimper (74) recognized that certain structures in the plasma do not necessarily originate from the undifferentiated plasma, but probably arise as the nucleus does by the division of preëxisting structures of the same sort. He called these bodies "plastids" and recognized three kinds: chloroplasts, leucoplasts, and chromoplasts.

A. Meyer (39) held that the plastids (trophoplasts) can develop into three different types of mature plastids: autoplasts (chloroplasts), anoplasts (leucoplasts), and chromoplasts. All three may contain starch inclusions. Crystalloids can develop in the anoplasts and autoplasts but are unknown in the chromoplasts. He agreed with von Mohl and Sachs in regarding the chloroplasts as naked, *i.e.*, without membranes. He says:

The strong refractive membrane which is apparent in the swelling of the protoplasm of the chlorophyll granule is an artifact and is not present in the intact cell. If it were an

originally present envelope of the thicker plasma, it would have to become more thin-walled in an expansion resulting from the swelling of the surrounding plasma or from endosmotic action, which thinning does not take place.

He also raised the question of the existence of a difference between the plasma layer next the chloroplast and the cytoplasm somewhat more distant. He reported that, when the chloroplast was spread out in a thin layer over a starch grain, it could be seen to consist of a clear spongy substance in which were enclosed at regular distances dark green granules of almost the same size. He was not able to determine whether or not the mass in which the granules were contained was colorless or only a very pale green. If the ground substance were completely colorless, these granules, which he named "grana," would contain all the chlorophyll. The granules were said to appear lighter toward the edge of the chloroplast.

Reinke (62) investigated the properties of the pigment chlorophyll both within the living plastid and in solution. He found no fluorescence whatever in the living or green plastid and assumed that there must be some sort of combination between pigment and plastid; for fluorescence was then held to be a property of solutions. He found chlorophyll fluorescent when dissolved in melted paraffin but not in the least fluorescent when the paraffin hardened. From Meyer's discovery that the grana swell when placed in water without losing their green coloring matter, he inferred that the grana are not composed of chlorophyll alone, but must contain some substance that swells in water, a material which is perhaps encased within the chlorophyll body itself. The next year, he (63) found a very weak fluorescence in the chlorophyll of living leaves which, however, was hardly comparable to the striking fluorescence of chlorophyll in solution. He also found that chlorophyll is weakly fluorescent in solid paraffin; about as fluorescent as in a living leaf of *Ficus elastica*. He still held that the chlorophyll must be combined with the ground substance of the plastid and must exist not as a solution but as finely divided particles.

Tschirch (91) revived the idea that the chloroplasts possess a membrane. The membrane, previously seen by Meyer and regarded as an artifact, he observed in the living cells of several species of aquatics. He says:

The chlorophyll granules of *Nitella* not only touch each other but are so closely pressed against each other that they flatten themselves even into polyhedral forms. Nevertheless the green tinted parts of the little grains do not lie beside each other but are separated by a hyaline zone of uniform width.

Schmitz (78) found in the chromatophores of the algae a fine fibrillar network such as Frommann had seen in phanerogams. He could not be certain whether this ground substance is green and the granules (or droplets) colorless, or the granules colored and the framework colorless. He did not believe that both scaffolding and droplets are colored, and was inclined to believe that the scaffolding is the colored part. Schaarschmidt (71) stated that: "In an optical cross section, the plasma coating shows very

well the hyaloplasmic membrane of the chlorophyll granules which was brought into question by A. Meyer." Schimper (74) held that in the simplest state the chromatophore consists of a colorless plasmic body which lacks every visible inner structure and inclusion and whose chemical nature agrees in the main with that of cytoplasm, from which it is differentiated by its greater density and its greater power of attraction for the coloring matter. The coloring matter was said to occur in the form of little droplets and to be only rarely crystallized. Grana were found by Schimper in the chloroplasts of all pteridophytes, phanerogams, and mosses; however, in all algae and in *Anthoceros* the chloroplasts in the living state appeared quite homogeneously green or finely spotted only on the outside.

Schwarz (79) stated his view of the structure of the chloroplast as follows:

I have reached the conclusion that a fibrillar structure belongs to the chlorophyll bodies, which however is not identical with that described by Schmidt and Frommann. The fibrils form in no way a reticulated net in which the junctures appear as intensely colored granules; the fibrils lie much more closely together, are less interlaced, fill up the whole mass of the chlorophyll body, and lie so closely together in the uninjured chlorophyll body that one is not able to determine with certainty their limits. They are bound together by an intermediate substance which is distinguished by its property for swelling and which can pass over into solubility. The separation of fibrils can be effected by a little soaking of the chlorophyll body or by causing a shrinkage of the fibrils by suitable reagents. Thus the differentiation of structural elements can be made possible. The fibrils are not equally colored but contain green colored vacuoles or globules which are identical with the grana of A. Meyer. The fibrillar substance is likewise colored, but to a small degree. The intermediate substance appears to contain no coloring matter. Fibrils and ground substance are chemically different protein bodies, for which I have proposed the names *chloroplastin* and *metaxin*. A chemically and morphologically different membrane is not present; it is likewise apparent that a so-called plasma envelope encloses the chlorophyll body.

Chodat (6, 7) agreed in general with the opinion of Pringsheim and Tschirch as to the spongy structure of the chloroplast. He held that the internal structure consists of a colorless stroma of a labyrinthine form surrounding irregularly shaped lacunae, which are themselves more or less subdivided by the lateral ramifications of the bands of stroma. The lacunae are more or less numerous according to the plastid studied. Their contour is always irregular. The edges of the bands of the stroma are always rounded. The chromatophores do not possess a membrane, as Tschirch had held, but what might be called a pseudomembrane (*Calanthe*). The fact that chloroplasts, when crowded together, do not form a solid green mass but are separated from each other by colorless zones, does not constitute a proof of a membrane, but shows only that the peripheral zone is not colored and is complete. In the colored chromatophores the walls (*Wandung*) of the lacunae are coated with a thin pigment layer.

Bredow (4) found that the ground substance of the chlorophyll bodies is not composed of fibrils. The transparent framework is very small-meshed with threads about the diameter of the meshes. In the latter

chiefly lie the coloring matter. Grana were nowhere found. Now and then oil droplets were found in the meshes, which very apparently had led to the hypothesis of granules. A plasma membrane around the chloroplast was well proved by the use of reagents in a clear majority of cases.

Kerner (23) held that the groundwork of the chlorophyll granules differs but little in structure and composition from the surrounding protoplasm.

They exhibit a pellicle-like, thickened outer layer; the inner portion, on the other hand, is formed of a porous mass of reticular or scaffold-like strands, which may be best compared to a bath sponge. The holes and meshes of this spongy, colorless ground substance contain a green coloring matter which is dissolved in an oily material, and clothes the continuous small spaces in the form of a parietal layer.

Meyer (40) reported that the shape of a chromatophore in which starch is being stored is to a certain extent determined by a differentiation of the pressure of the layer of the surrounding chromatophore on the starch grain. A thinner but more viscous layer of the chromatophore can exert more pressure. In addition he stated that: "A grain of starch contained in a constantly growing chromatophore receives on each part of its surface, in a unit of time, an increase, whose thickness is about proportional to that of the chromatophore layer which covers every point of the surface of the grain."

Timiriazeff (87), using Phajus, repeated the experiment of Nägeli of splitting the chloroplasts by bursting them in water. He arrested the process with dilute copper sulfate and stained the preparation with eosin. He obtained "a very elegant object, somewhat recalling the bud of a pink poppy with its bivalved calyx." He also examined the chloroplasts in red light and saw the grana as very small black specks in a very thin layer restricted to the surface. He found a strict measurement of the thickness of this layer to be out of the question, but estimated its thickness to be approximately one tenth micron (0.0001 mm.).

Küster (26) placed some leaves of *Sedum Sieboldii* in a weak nutrient solution for two or three days. He obtained and pictured shrunken and degenerating chromatophores surrounded by a fine membrane. He inferred that his treatment made the membrane encompassing the chloroplasts visible, but he could not be certain as to whether the membrane was a part of the chloroplast or an especially strong boundary layer of cytoplasm.

Wager (94) observed in certain cases a distinct fibrillar arrangement of the chlorophyll within the chloroplast. The green coloring matter appears granular when the chloroplast is in the epistrophe, fibrillar when it is in the apostrophe position. The fibrillar structure seemed to be that of fine fibrils lying more or less parallel, but a close examination shows that they are connected together here and there so as to give the impression of an elongated network. The granules are in fact so arranged and so numerous when the chloroplast is in epistrophe as to present a practically continuous



surface of chlorophyll to the action of light rays. The fibrillar arrangement, on the contrary, has numerous light spaces between the fibrils, so that less surface of chlorophyll is exposed to the rays of the light. A careful examination of the chloroplast in the epistrophic position renders it probable that the granular appearance is not due to the existence of separate granules of chlorophyll, but is probably an optical effect due to the superposition of alveoli one upon another, such as appears in fine oil foams. Wager was therefore inclined to the view that the chlorophyll corpuscle consists of a ground substance in the form of a delicate alveolar structure, in which the chlorophyll is more or less uniformly diffused. The diameter of the threads of this network is greater than in the apostrophe position, and this affords a means by which the chloroplast can accommodate itself to varying intensities of light.

Mereschkowsky (37) believed that the chromatophores are not organs which have gradually been differentiated from cell plasma. They are on the contrary foreign bodies, or rather foreign organisms, enclosed in the colorless plasma of the cell and existing symbiotically with it. They show a complete analogy with *Zoöchlorella*. A plant cell is thus nothing more than an animal cell with *Cyanophyceae* contained in it. The plants have thus been derived from the animals.

Priestly and Irving (57) studied the chloroplasts of *Selaginella Martensii* and *Chlorophytum elatum* by means of sections made with a freezing microtome. They obtained sections stated to be one micron thick, which showed that the green coloring matter is restricted to the peripheral layer of the chloroplast, which layer has a thickness of from one to three microns. In *Selaginella* this green ring showed no heterogeneity of structure, while in *Chlorophytum* a peripheral network was clearly indicated. Senn (80) found the colorless membrane of the chloroplast of *Funaria* especially evident when he placed the chloroplast in water at a temperature of 50° C. He saw pseudopods emerging from the region where swelling had first taken place. These pseudopods or plasma threads joined with one another. These threads were held to be responsible for the movement of the chloroplast, and, as they were organically connected with the colorless membrane which surrounded the stroma, it indicated that this membrane is not a mere boundary layer but a plasma sheath belonging to the chromatophore. As this sheath can send out pseudopods like an amoeba, and as it is a distinctly differentiated portion of the chlorophyll grain, he called it the "peristromium." This sheath is colored more intensely with iodine than the rest of the cytoplasm, but not so intensely as the stroma itself. Knoll (24) saw a peristromium in *Aspidistra*. It appears as a brightly lighted envelope, coating the completely green, finely clotted stroma. He later noted that in *Villaria* the cytoplasmic granules are outside of this "hull." He found that the protoplasmic strands (pseudopodia of Senn) are independent structures in no way connected with the sheath but merely adjoining it.

Küster (27) found that, if he plasmolyzed the epidermal cells of *Orchis latifolius* and later restored water to them, he could induce the leucoplasts to alter their forms in remarkable ways. As a result of his experiments he concluded that these leucoplasts are liquid and show a capacity for amoeboid changes of shape. He observed no peristromium in Senn's sense of the word. The pseudopods observed always belonged to the chromatophore mass itself, never to a plasmic organ surrounding it. These amoeboid changes in form did not, in general, affect the changes in position of the chromatophores.

Herlitzka (18), investigating the absorption spectra of chlorophyll in solutions of alcohol and acetone, in colloidal solutions derived from solutions in both alcohol and acetone, in the sap pressed out from the cells, and in the living leaf, found the absorption bands in the true solutions to correspond very closely with each other; there was also a close correspondence in the absorption bands between the colloidal solutions, solution in sap, and the living leaf. The absorption bands, however, in the living leaf, in the sap, and in the colloidal solutions occurred from eight to fifteen Ångström units nearer the red end of the spectrum than did the corresponding bands of the true solutions. He reported in addition that the sap solutions as well as the colloidal solutions of chlorophyll possess no fluorescent properties, and concluded that in the sap the chlorophyll is held in a solution of different form from that of its usual solutions with organic solvents; this form being that of a colloidal solution.

Rothert (64), following Schimper, recognized the similarity of the chloroplasts, leucoplasts, and chromoplasts and noted various intermediate forms between these types. He held that the chloroplasts and chromoplasts contain the same pigments but in quantitatively different proportions. He believed that the carotin and the chlorophyll are separated from each other in both the chloroplasts and the chromoplasts, the carotin being localized in the grana while the chlorophyll is distributed homogeneously throughout the whole.

Liebaldt (28) held that the chloroplasts of the higher plants consist of two phases, a hydroid phase and a green one of lipid character. He found no evidence of the existence of "grana." The chloroplasts seemed to be homogeneously green or at most somewhat finely granular. In the great majority of cases no definite special division of the two components (chlorophyll and ground substance) could be observed. This led him to accept the hypothesis that the lipid and hydroid phases exist as fine emulsoids. By treating with certain reagents he was able to separate the two phases until they became visible microscopically, the chlorophyll appearing as free colored droplets within the chloroplast.

Iwanowski (21), investigating the destruction of chlorophyll by light in solution of various concentrations, found that the more concentrated the solution the less susceptible it is to destruction by light. He came to the

conclusion that chlorophyll must exist in a colloidal form in the living chloroplast, for it is in the latter state that it is most indestructible. Chlorophyll in a true solution, no matter how concentrated, is much more easily destroyed than in the living leaf. On investigating the function of the yellow pigment in the chloroplast, he came to the conclusion that it has the important function of protecting the chlorophyll by absorbing certain blue and violet rays which are especially destructive.

Willstätter and Stoll (96) in their classic work on the chemistry of chlorophyll concluded that the chlorophyll in the leaf is held by adsorption to the colloidal ground substance of the chloroplast. Chlorophyll in the leaf has the identical absorption bands of colloidal chlorophyll, though there is a difference in the relative intensity of the various bands. Haberlandt (17) endorsed the view of Pringsheim, Meyer, and Schimper that the chlorophyll corpuscles of the higher plants are composed of a colorless matrix in which are imbedded the grana. He could not say whether or not the chloroplast is surrounded by a plasma membrane. However, in *Selaginella Martensii* he found a very distinct granular plasmatic membrane (3-4 microns thick) on the concave side of the chloroplast. Meyer (42, 43) concluded, through his studies upon *Tropaeolum majus*, that the "grana" are small oil droplets always to be met with in the growing chloroplasts. By treating the chloroplasts, bleached in alcohol, with Millon's reagent, he found them to be very rich in a protein substance (*ergastisches Organeisweiss*).

Stern (84), investigating the fluorescent properties of chlorophyll, came to the conclusion that chlorophyll fluoresces only when in a true solution; colloidal solutions of chlorophyll or solid chlorophyll do not noticeably fluoresce. As fluorescence was observed in the chloroplasts, he was of the opinion that chlorophyll as contained in the uninjured cell is in true lipid solution.

Mangenot (36) noted within the chloroplasts of *Vaucheria*, treated with a solution of osmic acid, a great number of globules stained brown which appeared as little brilliant points. These grew and formed a drop that remained attached to the chloroplast for some time. This oil he considered the first product of the photosynthetic activity of the chlorophyll of *Vaucheria*. Wurmser (97) found that by mixing a solution of chlorophyll, prepared by the method of Willstätter and Stoll, with various colloids, he could greatly retard the destructive power of light. He found a solution of gelatin to be more effective than either egg albumin, gum arabic, or starch. He concluded that the resistance shown by the chlorophyll in the living cell to the destructive action of light is due to its being protected by colloids. Meyer (44) held that each chloroplast is surrounded by a sheath of cytoplasm sharply differentiated from the rest of the cytoplasm by its lack of granules. This he called "metabolized cytoplasm," and he believed that it alone moves the chloroplast. This is the peristromium of Senn. It is homogeneous and is moved less easily than the normal granular

cytoplasm. Lubimenko (34), by grinding leaves of *Aspidistra elatior* with water in a mortar and filtering through clay filters several times, was able to get a clear green solution whose absorption spectrum was identical with that of the living leaf and quite distinct from the absorption spectra of chlorophyll *a* and *b*. He inferred from this that there is only one green material in leaves and that this is broken down by ordinary methods of extraction into chlorophyll *a* and *b* and yellow pigments. He is further of the opinion that in the plastid the chlorophyll is united chemically to the protein ground substance.

Scarth (70) concluded that under ordinary conditions the chloroplast of *Spirogyra* is an elastic gel. He inferred this from the facts: (1) that often, when the cell is plasmolyzed, the chloroplasts are too rigid to contract with the cytoplasm but maintain more or less nearly their original shape; (2) that the spiral form itself is one of minimal curvature and indicates elasticity. He also noted that electrolytes produced certain changes in the chloroplasts similar, in many respects, to their effect on colloids. An examination of a contracting chloroplast under the ultramicroscope gave a picture which represented different stages in the precipitation of an emulsoid. That the reaction occurred in a gel rather than in a sol mattered little, as the difference between these seems to lie only in the degree of approximation of their particles.

Lloyd (31-33), examining the chloroplasts in a number of living cells, was able to detect color effects which he interpreted as being attributable to a visible fluorescence of the chlorophyll when viewed ultramicroscopically. In contrast with the slight fluorescence shown by the chloroplasts of higher plants, the Cyanophyceae are highly fluorescent, which fluorescence he found to be due to their water-soluble blue pigment rather than to their chlorophyll. The evidence here brought forward seems to indicate strongly that chlorophyll does not exist in solution in the chloroplast, but is in some other form. Later in the same year he demonstrated that the chloroplasts of leaves and of green algae show a deep red fluorescence to a marked degree. Suspensions of living cells of *Scenedesmus* and *Menostoma* were fluorescent in light of wave length approximating  $530\text{ }\mu\mu$  and less. The same was true of suspensions of chloroplasts in solutions of cane sugar and in glycerin. Some of the chloroplasts (*Chlorophytum*) remained fluorescent in concentrated sugar solutions for over a month. In *Vaucheria* the fluorescent pigment soon became segregated into one or several vacuoles, which were individually fluorescent and which suffered more or less extrusion. There is a presumption that these vacuoles are identical with the "assimilatory substance" observed by Meyer and Mangenot. Lloyd held it probable that the granules which appear green in the chloroplast are really colorless, their green appearance, when observed within the chloroplast, being due to the green color of the surrounding stroma, while they themselves contain no chlorophyll. The non-granular stroma he finds to be the part of the chloroplast which emits fluorescent light.

## MATERIAL AND METHODS

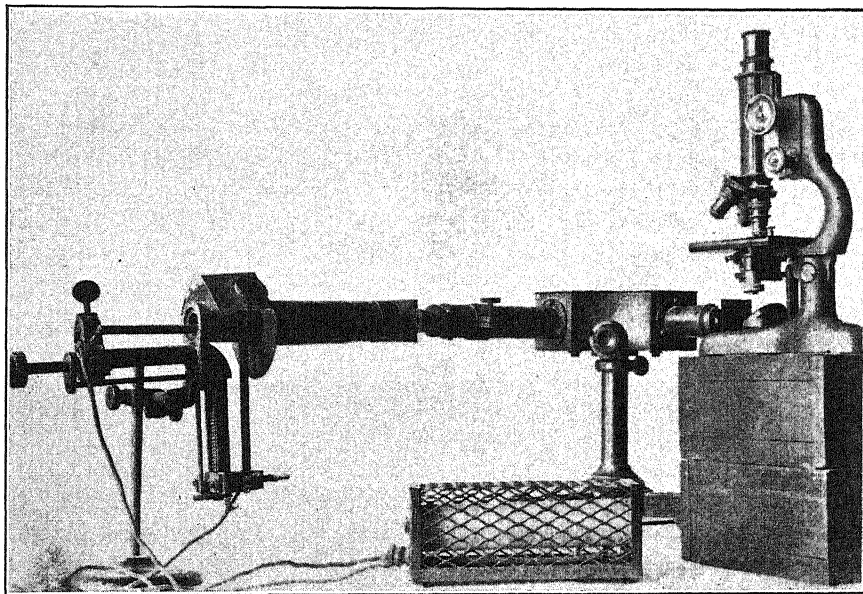
The usual technique of investigating the structure of a cell organ by fixing, sectioning, and staining it has never been very productive of results when applied to chloroplasts. Problems as to the physical state and distribution of the various pigments could not of course be solved by this method, for certain of the pigments are soluble in the dehydrating alcohol, some in the paraffin itself, while the water-soluble ones are generally extracted by the fixing fluid. Oily substances, occurring either as essential parts of the chloroplasts or as temporary inclusions, are removed during the imbedding process. The final result is that the chloroplast which is stained and examined is greatly modified, usually simplified. The physical condition of the ground substance must, of course, be much altered by fixation and dehydration. Hence it becomes necessary, if we are to determine the actual structure of the living, functioning chloroplast, to study it in its normal medium—the living protoplast.

Certain difficulties arise when we study the living chloroplasts by ordinary microscopic examination. The fact that they are quite small and contain pigments which conceal structural details has rendered results obtained by this method somewhat uncertain. The very divergent reports of different microscopists attest the inadequacy of this technique. We may, however, refine it somewhat by examining the chloroplast in *monochromatic light of a known wave length*. If we use a wave length which corresponds to the chief absorption band of a pigment, that pigment appears black in transmitted light, and all pigments not absorbing that particular part of the spectrum are invisible; for every pigment vanishes when examined in light of a wave length which corresponds to one of its bright bands. Thus it is possible to obliterate optically any or all of the pigments from a chloroplast without in any way altering the chemical or physical state of the ground substance. We are thus enabled to study the stroma of the chloroplast in the living condition without dissolving the pigments as in the methods hitherto used for this process, all of which entail the death of the entire cell. This spectroscopic method also enables us to examine the distribution of each of the various pigments in the chloroplast independently without subjecting the pigments to any conditions that might prevent their continued functioning. This method is but a slight modification of that used by astronomers for photographing separately the hydrogen, calcium, or other layers of the sun.

The apparatus used is quite simple (text fig. 1). It consists of an arc light, a monochromatic illuminator, and the usual mirror and condenser on the microscope. The light is directed into one arm of the illuminator. It passes down this arm to the angle of the two arms where it is split into the spectrum by two movable prisms. The light of the required wave length passes out through a slit in the end of the other arm to the mirror of the microscope, which reflects it up through the object examined. The

prisms are controlled by a screw so mounted that it is possible to read on a dial the wave length used.

For photographic work a Leitz "Micca" camera was used. Polarized light also was found very useful in examining the inclusions of the chloroplast. Its use made possible both the positive identification of a starch granule and the location of its hilum in relation to the enclosing body of the chloroplast. Iodine, when used for detecting starch, not only stains other structures beside starch but also "kills" the chloroplast.



TEXT FIG. 1. Method of illuminating the microscope with monochromatic light.

Living cells were examined with a Bausch & Lomb binocular microscope, using a 1.9-mm. oil-immersion objective. Nujol was found advantageous for examination of such water mounts in place of the more viscous cedar oil.

Certain organs to be investigated were of such a structure that, when whole, the chloroplasts could not be examined directly. Such structures were frozen and sectioned with a Bausch & Lomb freezing microtome. The sections were, as a rule, cut 5 microns thick, and these frequently contained sectioned chromatophores. Freezing, provided the temperature was never lowered below  $-4^{\circ}\text{C}.$ , alters chloroplasts in no observable way. Many aquatic plants, of course, may become imbedded in ice without injury, so it seems quite possible that a temperature of  $0^{\circ}\text{C}.$  or even slightly less actually causes no artifacts in the chloroplast. Temperatures below  $-4^{\circ}\text{C}.$ , however, apparently liquefy the chloroplasts, causing them to lengthen, to become spindle-shaped, and to fuse into a green mesh-work

that crudely resembles the chromatophore of *Chaetophora*. This observation corresponds with the results of Haberlandt (14). Of course, freezing and sectioning kill the cell, and probably only the cruder morphological characteristics of the chloroplasts remain unchanged. The pigments were not dissolved, however, as would be the case if the paraffin or the collodion method were used, and their distribution within the plastid could still be studied. As a check, the usual fixing and staining methods were also used.

Recently a number of investigators have been concerned with what may be called the "sub-microscopic" structure of the chloroplast. The questions of prime interest regarding the state of the chlorophyll as present in the chloroplast are: (1) Is it in a chemical or only a physical union with the ground substance? (2) Does it exist in a colloidal or in a true lipid solution? In order to "reach" the chloroplasts for any chemical tests, it was found necessary in the present work to remove them from the cell in as nearly an unchanged condition as possible. The cell contents were extruded into a fluid made to approximate cytoplasm in osmotic value, in viscosity, and in hydrogen-ion concentration. Lactose, sucrose, or dextrose was used to obtain the correct osmotic value; gelatin to increase the viscosity, and glycine (glycocoll) was used as a buffer to keep the pH constant. The most successful proportions were:

Lactose.....	0.2 mol.
Gelatin (sheet).....	2.0%
Glycine.....	0.03 mol.

The solution was kept at room temperature, 22° to 23° C. No observable change occurred in the chloroplasts when they were first placed in this fluid, and if kept in diffused light or darkness they remained unchanged for from 10 days to 2 weeks. At the end of this time bacterial growth destroyed the cultures. Thus treated, the chloroplasts were tested for lipoids, proteins, and certain carbohydrates, and were subjected to the action of certain enzymes—strepsin, pepsin, bromelin, diastase, etc.

The photostability of chlorophyll in different states varies enormously. Experiments were designed for measuring its photostability both in the "living" plastid and in the plastid which had been altered in various ways. By comparing the resistance of chlorophyll to destruction by light under various conditions, certain inferences were drawn as to its state within the plastid.

An examination of the chloroplasts of approximately 20 vascular plants revealed the fact that, except for certain aberrant types such as that found in *Selaginella*, they are of remarkable uniformity in structure. The variations observed are all relatively minor ones. This condition somewhat simplifies the presentation of the results here obtained, as it allows a type chloroplast to be chosen and described in detail, while the other chloroplasts studied need be described only in so far as they differ from this type. The chloroplasts of *Elodea canadensis* var. *gigantea* were selected as the type,

since they can be directly examined in the living condition and can easily be reached by reagents, the latter not being required to penetrate a thick cuticle as would be the case in the leaves of most terrestrial plants. Chloroplasts of spermatophytes investigated included those of *Cabomba*, *Ceratophyllum*, *Coleus*, *Dracaena*, *Ficus*, *Phajus*, *Pinus*, *Tradescantia*, etc. Among the archegoniates were *Adiantum*, both prothallus and sporophyte, moss protonema, *Pallavicinia*, and various *Marchantiales*. In addition, the chloroplasts of *Chara* and *Vaucheria* were investigated.

### RESULTS

One of the first problems attacked in this study was that of the nature of the boundary of the chloroplast. Numerous investigators have observed that, when two chromatophores come in contact with each other, the two green masses remain separated by a colorless hyaline zone. Tschirch (91) cited this fact to prove that the chromatophore possesses a membrane. Chodat (6) held that in *Calanthe* the appearance of this colorless zone is due to the fact that the outer layer of the ground substance is colorless. Senn (80) reported the formation of the pseudopods in the hyaline zone and called this zone the *perispermium*. Meyer (45) held that each chromatophore is surrounded by a non-granular layer of cytoplasm which he called the "metabolic" layer. The existence of a colorless zone about the chloroplast such as was noted by Tschirch and Chodat can be readily demonstrated in the living cells of *Elodea*. This colorless zone is clearly seen when an isolated chloroplast moves across the central vacuole, if it is examined in monochromatic light; for when thus examined the difference between the refractivity of this zone and that of the cell sap is not obscured by any chromatic aberration. It is also clearly visible as a light line between two chromatophores when they are in intimate contact. When the chloroplast is so placed that the outer limits of this zone are not clearly visible, its thickness can often be seen where it deflects a stream of moving cytoplasmic particles, or where cytoplasmic particles adhere to its outer surface. No granules in *sharp focus* were ever observed actually in contact with the edge of the colored portion of a chloroplast.

The thickness of this zone in *Elodea* varies from 1 micron to 0.25 micron and seems to be correlated with the state of the cytoplasm; for the zone is thicker when cyclosis is very sluggish and thinner when the cell contents are circulating rapidly. In fact, no distinct clear zone at all could be observed about certain rapidly moving chloroplasts, though evidence from other sources would indicate that one existed.

It is to be expected that chromatophores imbedded in cytoplasm should be covered by a non-granular layer. If we immerse a glass plate in soap suds, it will be covered completely by a layer of water, and the air of the bubbles will be separated by this layer from the glass. There is evidence, however, that this hyaline zone is something more than a mere adsorbed



layer of cytoplasm. It has a certain degree of permanency, for it has often been observed that the granules in a group adhering to this sheath keep their relative positions unchanged while the chloroplast is carried several times around the periphery of the cell. This would indicate that the chromatophore is not merely moving through non-granular cytoplasm, but rather that its covering layer remains with it. That the material of this layer, however, is not constantly associated with the chloroplast is indicated by the fact that its thickness is not constant. The physical state of this sheath is probably that of a viscous semi-solid, for granules adhering to its surface show no Brownian movement, no matter how active the other cytoplasmic granules may be. Often two or more chromatophores are so tightly held together by adherence of their sheaths that they will pass many times around the cell periphery before they separate or are pulled apart. Frequently, as they part, a strand of cytoplasm can be observed connecting them. Sometimes a strand of cytoplasm remains momentarily "hitched" to the sheath of a chloroplast and appears very much like a pseudopod of *Amoeba radiosa*.

It has long been a matter of dispute as to whether or not the chloroplast is surrounded by an organic membrane. Most of the early workers believed the chromatophores to be vesicles, and Nägeli (53) at one time even stated that each chromatophore is surrounded by a membrane of cellulose. Certain later workers, including von Mohl (49) and Meyer (45), did not admit the presence of a membrane about the chloroplast. Of more recent investigators, some have affirmed and some have denied that such a membrane exists. By "membrane" was meant a thin, firm layer which was probably conceived to be semipermeable. No such structure was observed in the present investigation. When the chromatophores were sectioned or torn to pieces mechanically, the outer layer appeared perhaps a trifle denser than the rest of the ground substance, although it could easily be seen that it was not a separate structure.

If we immerse a solid or semi-solid object in a colloidal sol with which it is miscible, a layer of colloidal particles will be adsorbed on its surface. This is what seems to happen when a chromatophore is immersed in cytoplasm, the colloidal particles of the latter being somewhat concentrated on the surface of the former. We recognize today that there are such things as liquid membranes, and there seems to be no reason for not considering this inner film of the cytoplasmic sheath as a membrane. The observed swelling of chromatophores in pure water or in aqueous solutions of low osmotic value does not seem to be due to any osmotic properties of this membrane, for that portion of the chloroplast which swells seems to be located within the ground substance.

Most of those who have studied the ground substance of the chromatophore are agreed that it is a protein. Mulder (51), indeed, considered it a wax derived from starch, and some of the other earlier workers thought

it to be starch. Recent workers, however, are agreed in thinking it protein. There is some dispute, however, as to its physical state. Küster (27) is of the opinion that the chloroplasts are liquid, while most other workers hold that the ground substance is semi-solid. Scarth (70) cites evidence indicating that in *Spirogyra* the chloroplast is an elastic gel.

The staining properties of the ground substance indicate that it is protein. Chloroplasts bleached by exposure to light or by alcohol are colored red with Millon's reagent and orange by the xanthoproteic test. The colors, however, are not as distinct as could be wished. In general, the reaction of chloroplasts to the histological stains shows that they are protein. The proteolytic enzymes, pepsin and bromolin, will digest them. That the ground substance is a gel rather than a sol can be demonstrated by teasing apart the chromatophores extracted from the cell. When ruptured mechanically the fragments maintain their shape. It is hard to conceive of a sol maintaining a definite structure such as the ground substance possesses in the living cell. The fusion of chromatophores at low temperatures is not conclusive proof that they are liquid. A frozen emulsion of linseed oil in water showed on examination the small oil globules fused into a meshwork shaped much like the chromatophores which had been reduced to a temperature of  $-10^{\circ}\text{C}$ . When the emulsion thawed, however, the droplets resumed their globular form. Chloroplasts, on the contrary, when once cooled to  $-10^{\circ}\text{C}$ . and then thawed, kept the form they assumed at the lower temperature. Pulverized agar, soaked in water until the grains had become rounded, was mounted in water on a slide and frozen. The agar particles also fused into a meshwork which persisted when the mount was brought back to room temperature.

The ground substance or stroma of the chromatophore in the living cell of *Elodea* is a hollow, flattened ellipsoid (Pl. XXI, fig. 1). The central vacuole within this stroma is, in a chloroplast which has been exposed to light, quite distinct. Its diameter is about half that of the chloroplast itself, and it contains, as a rule, a single large starch grain (fig. 2), though there may be as many as three grains in a single vacuole. The vacuole is visible even when no starch is present in the plastid, and can best be seen when the chloroplast is viewed edgewise in a strong beam of light (fig. 5). The vacuole is visible in quite young chloroplasts and does not seem to be a mere space left by the disappearance of an enclosed starch grain. The fact that starch grains slip out quite readily when the chloroplast is ruptured would indicate that they lie free in the vacuole and are not permeated by the ground substance. If an *Elodea* leaf whose chloroplasts contain small starch grains is immersed in boiling water for from 30 to 45 seconds, the chromatophores are ruptured by the swelling of the starch as this goes into solution, and their central vacuoles can then be seen even with the low power (16 mm.) objective (fig. 1). The chloroplasts now appear as green rings. If the chloroplast contains much starch, it will be entirely ruptured and the fragments of ground substance will appear as green hulls.

In cutting frozen sections of the leaf 5 microns in thickness, it frequently happened that a chloroplast was cut. No chromatophore was ever sectioned into three pieces, so no actual ring of stroma was seen, yet a careful examination of a chromatophore sectioned but once reveals clearly that it is a hollow sphere (Pl. XXII, fig. 9). Practically all who have investigated the chromatophore are agreed that the ground substance is spongy and contains many small granules in its meshes. There has been much dispute as to the nature of these granules. They have been reported as oil droplets, "grana," etc., and have been described by some workers as containing the coloring pigments and by others as colorless. When chromatophores in living cells are examined in strong white light, the peripheral region, which is made up of the ground substance, appears to contain numerous granules. These granules, though quite minute, having a diameter of from 0.25 to 0.5 micron, are of two distinguishable sizes. The smaller appear almost black when in sharp focus, while the larger granules appear colorless. When the cell is examined in light of from 500 to 550 Ångström units, it can be seen that the smaller black granules are not limited to the chromatophore, but are distributed throughout the cytoplasm (fig. 6). They are quite refractive in the light which passed through the chromatophore, but almost disappear in white light. Consequently they appear very distinct when they have a chromatophore for a background and practically vanish when their background is white. When the cell is examined in light of a wave length that is not absorbed by chlorophyll, these granules are as readily visible in the clear cytoplasm as when they have a chromatophore for a background. It was to be expected that investigators studying the living cell in white light only should have reported these granules to be a part of the chromatophore, especially as they often adhere so firmly to its cytoplasmic sheath that they may accompany a chromatophore several revolutions in its movement about the cell before they are carried off from it in the cytoplasmic stream. When the chromatophore is extruded from the cell, these granules rarely adhere to it.

The larger colorless "granules" mentioned in the preceding paragraph are quite visible in strong white light when the flatter surface of the chromatophore is in view. It is rather difficult to tell their exact distribution within the ground substance. They seem to be localized about the center of the broader surfaces of the chloroplast, for, when the chloroplast is viewed from the side, the ground substance appears homogeneous. These "granules" contain none of the pigments, for when the plastid is viewed in light of wave lengths corresponding to the absorption bands the granules still appear colorless (figs. 7, 8). These light "granules" are evidently not refractive oil droplets. Chromatophores in the cell and extruded ones also were tested for oil, but these "granules" were not stained either with Sudan III, osmic acid, or alkana. Neither were they colored by iodine, and they take no protein stains. The negative evidence derived from such

tests indicates that these apparent granules are really minute pores or vacuoles filled with something other than oil. They seem to be actual pores through the stroma, connecting the larger central vacuole of the chromatophore with the exterior. It is the fusion of these pores which makes the heated chromatophore ring-shaped. It is apparently the contents of these vacuoles and of the central vacuole which swell when the chloroplast is extruded into water. The fact that the swollen contents do not mix with water but are separated from it by a membrane indicates that these vacuoles contain something besides cell sap. Often in fixed and stained material the chromatophore has a granular appearance, but careful study shows that the apparent granules here are but the shrunken portions of the ground substance which in the living organism lie between these pores.

When the chromatophore of *Elodea* is teased apart, or when it is burst through contact with a solution of low osmotic value, it breaks into fragments which can best be described as knobby rods that contain the coloring matter. These fragments may be the fibrils described by Frommann (11), Schmitz (78), and Schwarz (79). If these rods lie so that their nodules are in contact, they form a reticulate structure of the sort described by Frommann and Schmitz. When they lie farther apart they seem parallel, a state which was reported by Schwarz who called the fibrils "chloroplastin." Schwarz could not recognize the chloroplastin fibrils in uninjured chromatophores, so it seems evident that these fragments are what he described. Nothing was found in the present investigation to indicate that these rods are represented by any definite structure existent in the living chromatophore. When fragmenting, the chromatophore breaks where it is weakest, *i.e.*, in the region of the pores, just as postage stamps separate along the lines of perforations.

Those investigators who have noted the granular appearance of the chromatophore are almost equally divided in their opinion as to whether the important pigments were located in the granules themselves or in the ground substance in which the granules were imbedded. Among those holding the former view may be cited Pringsheim (58), Meyer (39), Schimper (75), Haberlandt (15), and Timiriazeff (87), while among those inclined to the latter are Frommann (11), Schmitz (78), Schwarz (79), Bredow (4), and Wager (94). Lloyd (33) made the important observation that it is the non-granular part of the stroma which emits fluorescent light. Sachs (69) was inclined to believe that both granules and ground substance are colored, while Chodat (6) held that the lacunae within the chromatophores are lined by a thin pigment layer.

The opinion has been generally, though not unanimously, held that the pigments of the chloroplast are more or less restricted to the peripheral region. Timiriazeff even held that the pigments are confined to a surface layer but 0.1 micron in thickness.

The inclusions of vacuoles with reflecting surfaces and the accompanying

chromatic aberrations make it difficult to observe the exact locations of the pigments in the functioning chloroplast. When, however, the chloroplast is heated by immersion in boiling water and the vacuoles are thus caused to fuse (Pl. XXI, fig. 1), the stroma is easily seen to be homogeneously green. Thus it is shown that the ground substance under certain conditions may contain the pigments, though whether it does so in the living chloroplast is another question; for we can not be certain that the heating did not destroy whatever heterogeneity of structure the ground substance may have possessed.

When the shell of ground substance (the stroma) is examined in monochromatic light, its structure appears much more distinct. There is, of course, no chromatic aberration, and the fact that the pigments can be made to appear black against a colored background makes it possible to locate them with much more definiteness. The examination of chloroplasts in light of various wave lengths, corresponding to the absorption bands of each of the different pigments, revealed the fact that the pigments are intimately mixed and evenly distributed throughout the ground substance. The only parts of the chloroplasts not colored were the pores and the starch inclusions. The pigmented ground substance appeared black in light of from 660 to 690 Ångström units and from 470 Ångström units on through to the end of the spectrum. The absorption band in the red corresponds to that observed by Herlitzka (18) in the living leaf. This band is roughly 16 units to the left of the corresponding absorption band of chlorophyll *a* and about 30 units to the left of the band of chlorophyll *b*.

#### EXPLANATION OF PLATES

These photographs were taken with a Leitz "Micca" camera. The prints were enlarged to a diameter of twice that of the negatives. Unless otherwise stated, the magnification is 960 times. None of the photographs are retouched.

##### PLATE XXI

FIG. 1. Chloroplasts of *Elodea* which have been boiled 15 seconds. The central vacuole in each chloroplast is left clearly visible by the included starch going into solution.

FIG. 2. Chloroplasts of *Elodea* stained with iodine, showing starch inclusions.

FIG. 3. Chloroplasts of a fern prothallus photographed in light of 550 Ångström units. The chlorophyll has been removed optically, with the result that the chloroplasts appear of the same color as their background.

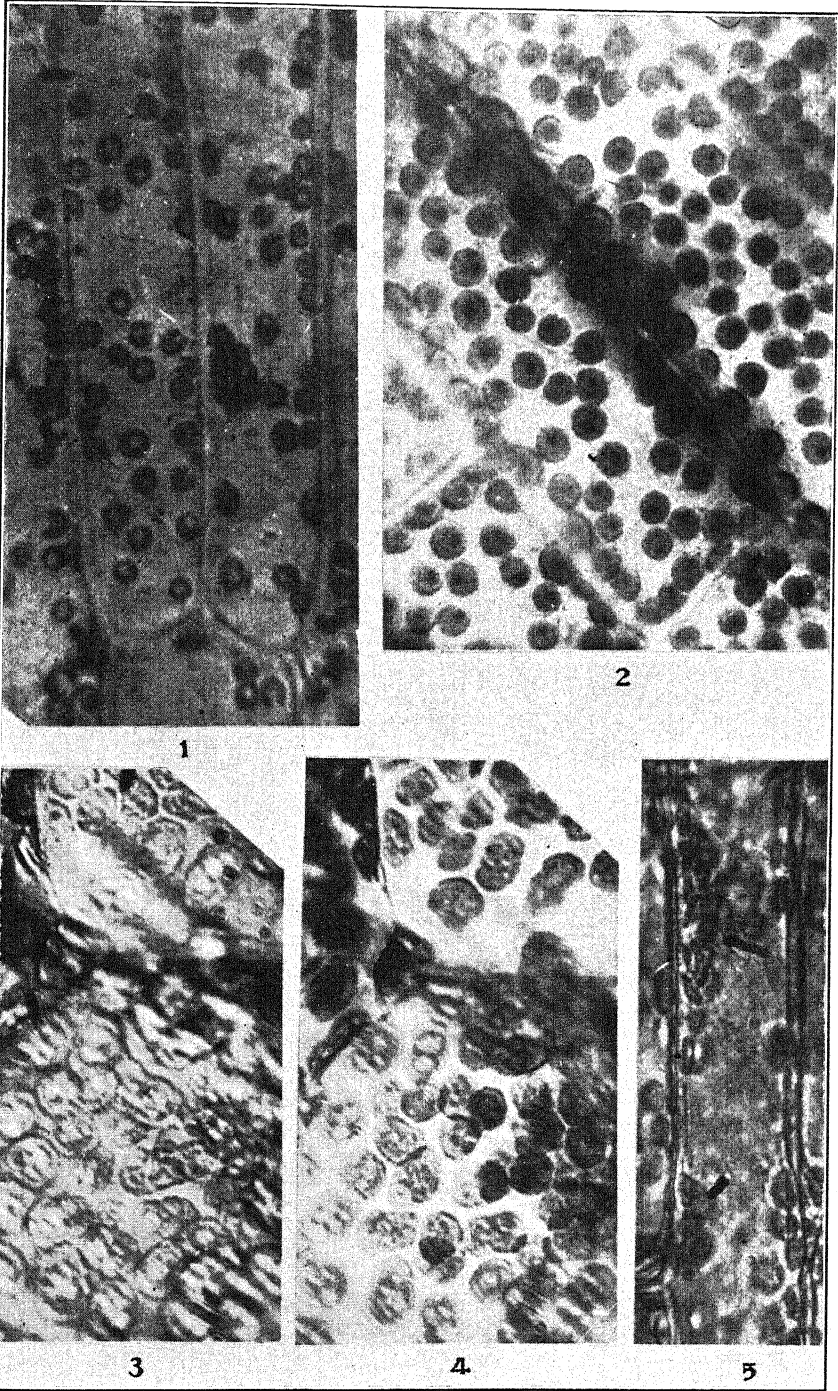
FIG. 4. Same as figure 3, photographed in light of 450 Ångström units. Chlorophyll appears black in this light, so that the chloroplasts appear distinct from their background.

FIG. 5. Living cell of *Elodea*. The central vacuoles are visible in certain chloroplasts seen on edge.

##### PLATE XXII

FIGS. 6-8. Chloroplasts of *Elodea* (living) in light of 550, 450, and 400 Ångström units, respectively. Figure 6 shows the cytoplasmic granules, which are quite invisible in figure 8.

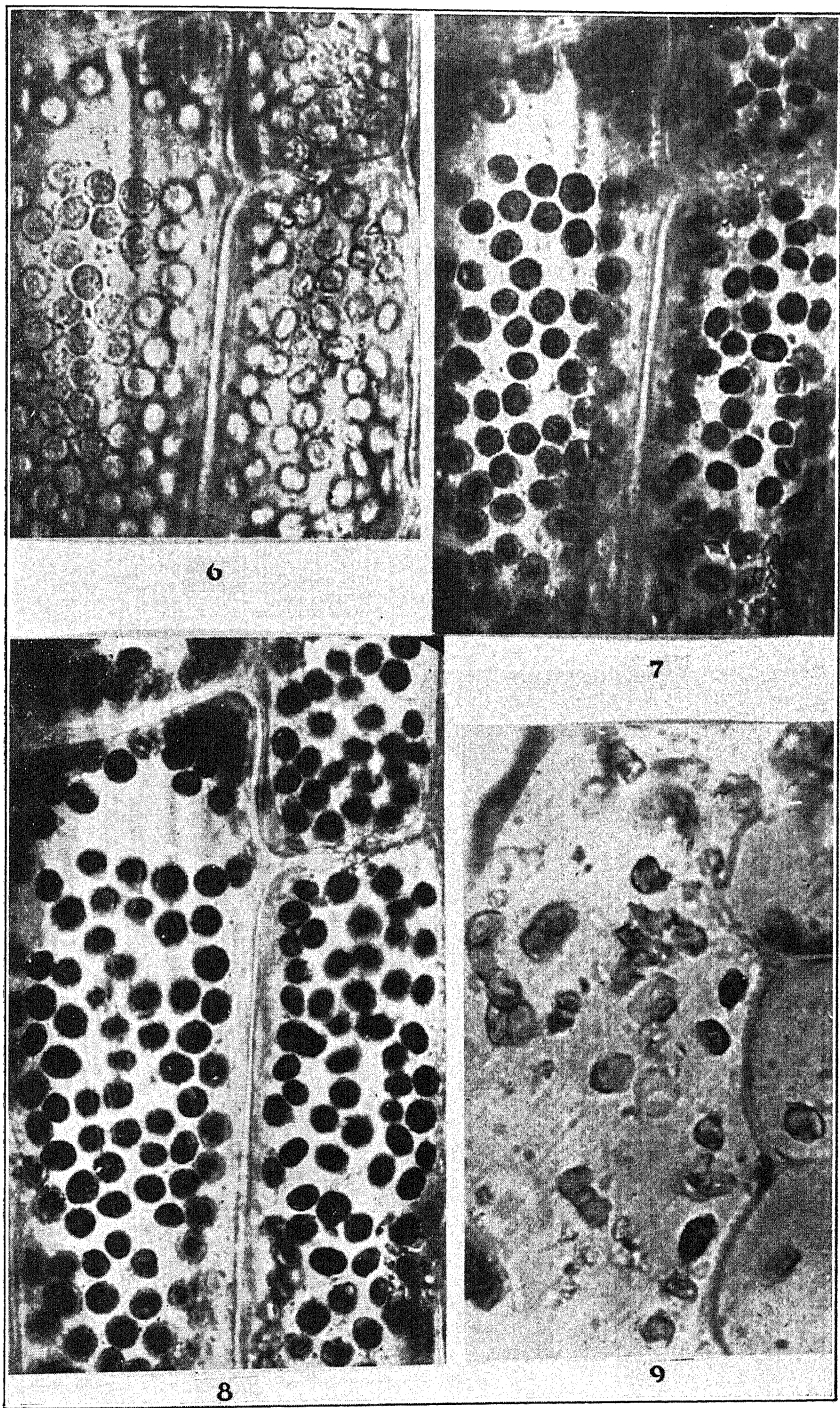
FIG. 9. Fragments of chloroplasts of *Marchantia* sectioned with a freezing microtome. The sections were approximately 5  $\mu$  in thickness.



ZIRKLE: STRUCTURE OF CHLOROPLAST







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## THE STRUCTURE OF THE CHLOROPLAST IN CERTAIN HIGHER PLANTS

### PART II

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It is clear from what has been said in the previous part (p. 312) that many features of the microscopic structure of the chloroplast can really be determined by direct observation. A very real problem, however, whose solution would perhaps be a great help in accomplishing the artificial synthesis of carbohydrates, is the determination of the state of the chlorophyll within that part of the plastid which appears homogeneously green. Unfortunately this "sub-microscopic" structure is not disclosed by a direct examination and can at present only be inferred from somewhat incomplete and seemingly contradictory evidence. The various workers who have investigated this problem have arrived at quite different conclusions.

Liebaldt (28) and later Stern (84) concluded that the chlorophyll in the living plant exists in a true lipid solution. Iwanowski (21), as a result of investigating the destructive power of light upon true solutions, and Hertizka (18), through a study of the absorption bands in the spectrum of chlorophyll in various states, were led to believe that chlorophyll exists in the leaf as a colloid. Wurmser (97) showed that chlorophyll could be protected from destruction by light by mixing it with a colloid, and concluded that it is so protected in the functioning chromatophore. Willstätter and Stoll (96) were of the opinion that chlorophyll is adsorbed upon the colloidal particles of the stroma, while Lubimenko (34) asserted that it is held in chemical union with the ground substance.

The fact that chlorophyll is lipid-soluble has raised the question as to whether or not it exists in such a solution within a chloroplast. Liebaldt held that the plastid consists of two phases, a colorless hydroid one and a lipid one, which latter alone contains the dissolved pigments. By treating the chloroplast with various alcohols he caused the pigments to become localized in droplets large enough to be observed microscopically. These droplets seemed to be insoluble in water and were looked upon as the lipid solution which, before contact with the alcohol, had existed as a sub-microscopic emulsoid. Such droplets adhering to the chloroplasts are shown in

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Plate XXIII, figure 13. Since Willstätter and Stoll have shown, however, that certain alcohols react chemically with chlorophyll by replacing the phytol (phytyl alcohol) portion of the chlorophyll molecule and forming a chlorophyllide, Liebalddt's views are open to several interpretations. Phytol ( $C_{20}H_{39}OH$ ) is a colorless, viscous oil. The green droplets observed by Liebalddt are doubtless lipid, but it is possible that the lipid is not one which occurs free in the plastid, but one which was produced by the treatment to which the plastid was subjected. It may well be that these droplets are merely phytol containing methyl-, ethyl-, or propyl-chlorophyllide, etc., in solution. The probability of this suggestion is increased by the fact that when chloroplasts are treated with acetone the coloring matter does not collect in droplets but diffuses out into solution. Green droplets can be formed by treating the acetone-chlorophyll solution with water, but this merely precipitates the chlorophyll out of solution.

When chloroplasts are tested for oil the results are quite inconclusive. Alkana does not stain them and osmic acid colors them but slightly. Extruded chloroplasts are distinctly colored by Sudan III (anilin red dissolved in ethyl alcohol), but this is no real test for an oil in this instance, for the test itself (the solvent of the dye) produces an oil (phytol) and naturally the result is positive. Anilin red stains hydrocarbons, glycerine esthers, waxes, etc. Hence we should expect chlorophyll itself to be stained, which apparently is what happens when the chloroplasts are treated with the dye dissolved in acetone. Chloroplasts from which the pigments had been extracted with alcohol (methyl-, ethyl-, or butyl-alcohol), or with acetone, or in which the pigments had been destroyed by light, were not colored when treated with anilin red. The yellow chloroplasts of wheat seedlings sprouted in the dark, however, were stained when so treated.

Chlorophyll, in all cases in which it is known to be in a lipid solution, is very easily destroyed by direct sunlight. Its stability seems to increase with the strength of the solution, yet very dense solutions can be destroyed in a few hours' time. If therefore the chloroplasts contain chlorophyll in a lipid solution they should bleach quite rapidly when placed in direct sunlight. But this is by no means the case. Chloroplasts were extruded from a cell of *Elodea* into a solution containing 0.2*M* cane sugar, 2% gelatin, and  $\frac{1}{4}$ % glyocol and placed in direct sunlight in a greenhouse. During an exposure of two weeks they showed no trace of bleaching. At the end of that time they were destroyed by bacteria. Chlorophyll in the unpoisoned plastid then is very stable.

Under certain other conditions chlorophyll is photostable in the killed and fixed plastid. Sprigs of *Elodea* were fixed by boiling, by treating with formalin, or with bichlorid of mercury, or, finally, by immersion in pure glycerin without previous treatment. Each sprig was cut in two, one half being placed in a vial of water and the other in a vial of pure glycerin. The vials were then placed in direct sunlight in a greenhouse. The sprigs which

had been placed in water were completely bleached by the end of the first day; those in glycerin showed no sign of bleaching after four months' exposure. The fact that the chlorophyll in the plastid is photostable in pure glycerin has an important bearing on the question as to its state in nature. If the chlorophyll existed in the plastid dissolved in sub-microscopic droplets of oil the glycerin should not prevent its destruction, as neither chlorophyll nor oil is soluble in glycerin. A solution of chlorophyll in a fine emulsion of glycerin and nujol was completely bleached by a four-hour exposure to sunlight. The glycerin seems to preserve the chlorophyll by removing all water. The evidence for this will be presented later. It may be noted here also that a sprig of *Elodea* immersed in melted dextrose keeps its color in direct sunlight for weeks, while a leaf that had kept green in glycerin for several months lost its color in a few hours after it had been replaced in water. The results of an experiment upon *Vaucheria* to be described later also make it seem extremely improbable that chlorophyll exists in a lipid solution in the plastid.

The chief evidence for the existence of a chlorophyll-lipoid solution in the plastid consists of the latter's fluorescent properties. Chlorophyll has thus far been demonstrated to be fluorescent to a marked degree only when in a true solution, and Stern (84) noted that many chloroplasts are markedly fluorescent. Lloyd (31), by means of a new and refined technique, was able to demonstrate this fluorescence.

If the pigment in the chloroplast could be shown to be in a colloidal, rather than in a true lipid solution, the significance of a number of observations would be clear. The absorption bands in the spectrum of a living leaf are those of colloidal chlorophyll and not those of a lipid solution of chlorophyll. The fact, first noted by Willstätter and Stoll (96), that water-free solvents such as benzene, ether, acetone, absolute alcohol, etc., in which extracted chlorophyll is soluble, will not extract chlorophyll from dried leaves unless a little water is added, while they will extract it from fresh leaves, is best explained by the hypothesis that chlorophyll is held in the plastid in a colloidal state. When water is added to the dried leaves, the contained mineral salts form a solution which alters the colloidal state of the chlorophyll and renders it soluble. A dried colloid, also, would have much less surface upon which solvents could act than would one whose particles were dispersed in water.

Chlorophyll resists destruction by light in the absence of water as it does to going into solution under like conditions, as was shown by the following experiment. A sprig of *Elodea* was dried in an electric oven for 12 hours at 106° C. It was then cut in two, when one half was placed in a desiccator and the other half in a moist chamber. Both were placed in direct sunlight. The half in the moist chamber was entirely bleached by the end of the first day, while the half in the desiccator showed no sign of bleaching after an exposure of three weeks.

A series of solutions of water and glycerin were made, ranging from 10% to 90% glycerin with 10% intervals. Elodea sprigs which had been killed by boiling were placed in each solution, and then all were placed in direct sunlight. At the end of the first day the sprigs in the solutions containing 50% of glycerin or less were completely bleached. The one in 60% glycerin was somewhat bleached, and the one in 70% glycerin was slightly less faded. The sprigs in 80% and 90% glycerin had lost no color. At the end of the second day, however, the sprig in the 60% glycerin was completely bleached, the one in 70% was about half bleached, and the one in 80% had started to fade. At the end of the third day the sprig in 70% glycerin was completely faded and the one in 80% had appreciably lightened in color. At the end of a week the Elodea sprigs in all solutions including the one in 90% glycerin were completely bleached, while the control, a sprig in pure glycerin, had lost no color after a four months' exposure. The loss of color was thus slower in those solutions which contained little water, although the bleaching finally went as far in them as in the others. Another similar series of solutions was made of glycerin and 95% ethyl alcohol, and an Elodea sprig was placed in each. Fading was more rapid in those solutions which contained little glycerin than in the corresponding solutions of glycerin and water. The Elodea in all solutions which contained 70% glycerin or less faded at the end of the first day. The chlorophyll, however, in the solutions containing 85%, 90%, and 95% glycerin (with 15%, 10%, and 5% respectively of alcohol) was quite photostable and had lost little or no color after a two weeks' exposure. Thus, in spite of the fact that chlorophyll is more easily destroyed by light when in an alcoholic solution than in a colloidal solution in water, it is *less photostable* in the *presence of a little water* than in the *presence of a like proportion* of alcohol. Elodea, immersed in methyl-, ethyl-, or butyl-alcohol, glycol, acetone, petrol ether, nujol, or in any liquid in which chlorophyll is soluble, was very quickly bleached. It could not be urged that the chlorophyll was destroyed by chlorophyllase, which required a trace of water for it to act, for when any chlorophyllase which might be present was destroyed by heat the reaction was not altered.

The question as to whether the pigments are united chemically or merely physically to the ground substance can not yet be definitely settled. Lubimenko's (34) observation that in *Aspidistra* the green pigment was quite photostable in an aqueous solution as long as the accompanying proteins were not coagulated does not necessarily mean that its stability in the living leaf is due to a chemical union with the protein of the plastid; for Wurmser has shown that the resistance of chlorophyll to destruction by light can be greatly enhanced by merely mixing it with a colloid. Iwanowski (22) concluded that the yellow pigments in the plastid absorb certain of the light waves which are most destructive to chlorophyll and thus help to keep it photostable in the plastid. Chlorophyll in plastids extruded from the cell, but whose ground substance was not coagulated either by heat or by

chemicals, the author found to be photostable until the plastids were destroyed by bacterial action. This stability was lost as soon as the plastid was "poisoned."

Whatever the union between the chlorophyll and the ground substance may be, it is not a very strong one, as it can easily be destroyed by many solvents. The union does not seem to affect any of the properties of chlorophyll—its staining qualities, solubility, or its reactions to acids, bases, etc.—while the presence of chlorophyll within the plastid does alter certain properties of the latter. Bleached or albino chloroplasts are digestible by the usual proteolytic enzymes. Extruded green chloroplasts could be digested by neither pepsin, papain, nor bromelin. The addition of a lipase (steapsin) had no appreciable effect. A possible explanation of these facts is that the chlorophyll is so distributed on the surface of the colloidal particles of the stroma as to prevent the enzymes from reaching the protein ground substance. The particles are, of course, so small that if the pigments were thus distributed the stroma would appear homogeneously green. Certain staining properties of bleached and unbleached chloroplasts add to the probability of this explanation.

Both green and white chloroplasts were colored equally readily by certain stains. They reacted differently, however, when treated with neutral red. Green plastids, whether within the cell or extruded, were not in any way colored by this stain, although, if first bleached with acetone, methyl-, ethyl-, or butyl-alcohol, they were stained a deep red. No matter how the plastid was "fixed," whether by boiling, formaldehyde, etc., it would not take the stain so long as it was green. Even if the plastid was treated with a chlorophyll solvent, it remained unstainable as long as it contained any visible chlorophyll. So it does not seem probable that the staining capacity of the bleached chloroplast is due to the chlorophyll solvent acting as a mordant. This seems especially improbable in view of the fact that chloroplasts bleached by sunlight readily took up the stain, and in a strain of variegated *Tradescantia* the albino chloroplasts were readily stained in the living cell while the green ones were not. The yellow chloroplasts of wheat seedlings grown in the dark were impervious to the stain.

The hypothesis here suggested to account for the differences in behavior of green and bleached chloroplasts when treated with proteolytic enzymes or with neutral red is in no way invalidated by the fact that both types of chloroplasts are equally colored by various stains such as methylene blue, safranin, etc. It may well be that our hypothetical chlorophyll layer is permeable to some stains and impermeable to others. The evidence here presented can best be interpreted by assuming that chlorophyll exists in the plastid in a colloidal state and covers entirely the colloidal particles of the ground substance. This is essentially the view presented by Willstätter and Stoll (96). The problem is by no means settled, however, for the suggested hypothesis does not explain the observed fluorescence of the functioning chloroplast.

It has long been known that those plastids whose main function is the assimilation of carbon and those in which the prepared food is stored carbohydrate are closely related. In fact, there is no hard and fast distinction between the two types, for on occasion each can acquire the form and color and perform the function of the other. The leucoplasts near the surface of a potato tuber become green chloroplasts when the potato is exposed to light, and certain chloroplasts, when shaded, lose their color yet retain their stored starch. Under normal conditions, however, these two types are quite distinct. There are certain plastids, occurring in both stem and leaf of *Elodea*, which perform the function of both chloroplast and leucoplast. The normal chloroplast of the *Elodea* leaf is concerned primarily with synthesizing carbohydrates, and it stores the manufactured food in itself only temporarily. Its contained starch will generally disappear within one or two days if it is so placed that it can not manufacture new starch (Pl. XXIII, fig. 10). There are, however, specialized chloroplasts in this transparent leaf whose primary function appears to be the storage of starch and whose photosynthetic activity is a distinctly secondary one (figs. 11, 12). These specialized plastids are not leucoplasts, however, for they contain abundant chlorophyll and are fundamentally the same in structure as the ordinary chloroplasts, from which they differ apparently only in size and in the relative proportion of the vacuoles and strands. They contain, as a rule, but a single starch grain, though at times as many as three grains are present. They retained this stored starch as long as the leaf could be kept alive in the dark. This differentiation of the chloroplasts was also observed in several genera of the Marchantiales and in the green stems of numerous seed plants. It seems to be quite generally distributed.

These specialized chloroplasts in the leaf of *Elodea canadensis* var. *gigantea* are localized in the two layers of parenchymatous tissue which surrounds the midrib (Pl. XXVII, fig. 44). In the smaller form, *Elodea canadensis*, they occur in numbers in the basal, dorsal cells of the leaf. The cells of the outer layer are somewhat larger than those of the inner one, and a like difference of size is also found in the contained chloroplasts, those in the outer layer often being 25 microns in their greatest diameter while those in the inner rarely exceed 12 microns. Even the smaller of these specialized chloroplasts have a volume approximately 8 times as large as that of the chloroplasts in the other cells of the leaf. Most of this bulk is taken up by the enclosed starch, the ground substance being limited to a thin, vesicle-like peripheral layer about 2 microns in thickness. The green color of these chloroplasts is noticeably paler than that of the ordinary ones, and it is estimated that they contain the same amount of chlorophyll as the latter but that their colored portion is stretched out to a thinner layer.

These specialized chloroplasts of *Elodea* are located in cells whose cytoplasm is more responsive to the stimulation of light than that of the ordinary photosynthetic tissue. Thus, when a leaf in which there is no observable

cyclosis is stimulated by the light from a carbon arc, the resulting cyclosis can first be observed by the movement of these specialized chloroplasts, which respond to the stimulus in from  $\frac{1}{4}$  to  $\frac{1}{3}$  of the time required for the ordinary chloroplast to react. Boiling, which causes the ordinary chloroplasts to become ring-shaped, causes the specialized ones to fragment, the fragments which still contain the pigments being evidently pieces of a hollow spheroid.

A like differentiation of chloroplasts occurs in the *Elodea* stem. The chloroplasts in the epidermis are indistinguishable from those in the normal chlorenchymous leaf tissue. As shown by figures 41*a-e*, the chloroplasts are larger and lighter in color as their position nears the central cylinder, the largest occurring in the endodermis. Indeed, in frozen and sectioned material the endodermis contained a few starch grains without visible covering. It seems, however, that these starch grains were contained in the living cell within the chloroplasts which were ruptured by the sectioning. The chloroplasts in the tissue between the epidermis and the endodermis show numerous intermediate stages between those of the bounding tissues. The relative sizes of the chloroplasts are shown in figure 41 *a-e*. The size of each chloroplast seems to be determined by that of the contained starch grain, and by altering the latter experimentally the size of the former can also be altered. Shading the *Elodea* or placing it in direct sunlight appears not to alter the size of the included starch grain and consequently of the enclosing chloroplasts of either the epidermis or the endodermis, the plastids at the two ends of the series in size. The intermediate plastids, however, are materially altered. When the plant is shaded, they approach in form the chloroplasts in the epidermis; when the leaf is exposed to direct sunlight, their included starch grains enlarge and the chloroplasts resemble more closely in form those in the endodermis. The size of the plastids is not directly affected by the light, for chloroplasts in cells equidistant from the center of the stem are approximately equal in size, being as large on the shaded side as upon the side exposed to the light.

#### CABOMBA, CERATOPHYLLUM

The chloroplasts of these aquatics are essentially like those of *Elodea*. As the chloroplasts of the former can be clearly observed only when the tissue which bears them is sectioned, it can not be stated definitely that they were investigated in an unaltered condition. Although no sheath could be positively demonstrated in this case, it is probable that these chloroplasts are imbedded in a sheath of non-granular cytoplasm, since all chloroplasts which were observed in living cells were so imbedded. The radial pores through the stroma of the chloroplasts of *Cabomba* and *Ceratophyllum* are a trifle more distinct than those of *Elodea*, and consequently the stroma appears more granular. A real difference exists, however, between the chloroplasts of *Elodea* and those of the other two aquatics in the location of



their starch inclusions. The starch grains at first are initiated within the central vacuole just as in *Elodea*. They grow by accretions laid down on all sides, for, when mature grains are examined in polarized light, the hilum can be observed to be centrally located. As the starch grains increase in size they become excentric in the chloroplast and are enclosed by a thin layer of the green stroma while the mass of the chloroplast lies off to one side (Pl. XXIV, fig. 18; Pl. XXVII, fig. 43). The starch never appears actually to protrude from the ground substance, but remains enclosed always by a thin green layer which is continuous with the rest of the chloroplast. It is interesting to note that the starch grain is always in that side of the chloroplast which is nearer the center of the cell, no matter from what direction the light may come. Thus the light, before it reaches the starch grains in approximately one half of the chloroplasts, passes through the mass of the chlorophyll-bearing stroma and through the starch grains before reaching the mass of the stroma in the other half. The diameter of the chloroplasts in the epidermis of the leaves of *Ceratophyllum* and *Cabomba* is the same in both, but is only half the diameter of those in the chlorenchyma of these genera.

#### THE CHLOROPLASTS OF THE FERN PROTHALLIUM

The chloroplasts in fern prothallia appear almost exactly like those of *Elodea*. The sheath of non-granular cytoplasm which surrounds each one can be very easily demonstrated, for the chloroplasts frequently become so crowded as to become polyhedral, yet a colorless hyaline zone is always evident between the green masses of two adjacent plastids. The stroma here often appears much less granular than that in *Elodea*, for the pores are less conspicuous and at times can not be observed at all. The central vacuole can easily be seen in the living chloroplast even when there are no starch inclusions, and there is no mistaking it when it contains, as frequently happens, two or three minute starch grains. Sometimes a larger chloroplast has two vacuoles (Pl. XXIII, fig. 14) each containing a single starch grain.

It has long been known that chloroplasts have become adapted to the osmotic pressure of their cell sap. The cell sap of a fern prothallium, as shown by the plasmolytic method, has a higher osmotic value than that of *Elodea*. As was to be expected, the fern chloroplast, when extruded into tap water, swells more than one from *Elodea*. Nägeli (54) first noticed this swelling, and his observations have been confirmed by Timiriazeff (87), Priestly (57), Liebaltd (28), and others. Schwarz (79) held that it is the colorless portion of this chloroplast, the "metaxin", which is responsible for the swelling. The present investigation has disclosed the fact that it is not the stroma itself that swells, but substances within the pores and the central vacuole. Figure 17 (Pl. XXIV) shows the distended contents of the central vacuole. The stroma had been ruptured and remains adhering to the now bladder-like vacuole. The surface film at the interphase of vacuole and water is quite distinct. This film has doubtless been observed and has been

reported to be the membrane which surrounds the chloroplast. A close examination will indicate, however, that the stroma is located on the outside of this film. Other examples of this swelling will be cited under *Phajus*. Attempts to stain the contents of these vacuoles did not succeed. The probabilities are that they contain a little sugar in watery solution with enough dissolved protein to hinder its ready diffusion out into the surrounding water when the chloroplast is extruded from the cell.

Little difference can be observed between the chloroplasts of the fern gametophyte and those of the sporophyte. In a number of instances the neighboring chloroplasts in a living and apparently normal cell of the gametophyte are fused into chains. This has not been observed in cells of the sporophyte. Figure 16, Plate XXIII, shows the chloroplasts of a cell in a prothallium which has been fixed by immersion in boiling water. Figure 15 shows the chloroplast of a cell in the first leaf of the attached sporophyte. Both cells were, of course, treated exactly alike. The difference shown here, however, is not a universal one. The chloroplasts of the liverworts studied were like those of the fern gametophyte. Figure 23, Plate XXIV, shows some chloroplasts of a *Pallavicinia* thallus that had been heated to boiling. The fragments of the chloroplasts, ruptured by swelling of the starch as it went into solution, appear as pieces of a hollow spheroid. The central vacuole of the chloroplast of *Conocephalum* appears quite distinct in figure 20. In this and in other *Marchantiales* the chloroplasts deep within the thallus contain more starch than those near the upper surface.

#### THE CHLOROPLASTS OF PHAJUS AND OTHER PLANTS

The uniformity of the chloroplasts in the leaves of the Spermatophyta has been frequently noted. Möbius (48) found that the chloroplasts of approximately 75% of the species he studied had a diameter of between 4 and 6 microns. In the present investigation no appreciable differences were observed between the chloroplasts in the foliar tissues of plants as far apart systematically as *Phajus*, *Triticum*, *Tradescantia*, *Vallisneria*, *Aponogeton*, *Dracaena*, *Ficus*, *Ranunculus*, *Pinus*, *Psilotum*, etc. The chloroplast of the leaf of *Phajus* will be taken as a type of this group and, as it is of the same general type as that of *Elodea*, its characters will be described in detail only as it differs from the latter.

The chloroplasts of *Phajus* appear to be quite granular (fig. 19). This appearance is caused by the size and prominence of the pores which connect the central vacuole with the surface. Indeed, in some instances when the chloroplast is swollen, the stroma with its contained chlorophyll appears to be ruptured in the regions between the pores, and the chloroplast then seems to be made up of green "grana" imbedded in white "metaxin". The central vacuole at certain times contains no visible inclusions, at other times it contains a single grain of starch (fig. 21). No starch except that in the central vacuole was ever observed about the chloroplasts. The osmotic

value of the cell sap of *Phajus* seems to be greater than that of fern prothallia, for the chloroplasts when extruded into water swell more than do those of the fern. The swelling substances of the central vacuole do not, as in the fern (fig. 17), break through the stroma in a single place and swell out to the side of the chloroplast, but regularly the stroma becomes ruptured in numerous places and distributed over the surface film of the vacuolar contents (figs. 22, 24). At times, though rarely, the stroma does not rupture and the contents of the central vacuole extrudes through several of the pores and remains in the form of bubbles attached to the stroma. In one instance, when the chloroplasts of *Aponogeton* were being examined in water, the contents of the central vacuole of a chloroplast exuded through a single swollen pore, rounded up, and floated away from the plastid.

In certain cells in the leaf of *Pinus*, and also in the protonema of a moss, the presence of oil droplets made possible an experiment described in detail under *Vaucheria*. In the leaf of *Peperomia hispidula* there are two distinct types of chloroplasts (Pl. XXVI, fig. 34); the larger one, which occurs in palisade cells, contains many minute pores in the stroma (Pl. XXVII, fig. 43) while the smaller one seems to be little more than a hollow spheroid which contains a starch grain.

In the tuber of *Phajus*, the development of the chloroplasts is in many ways unlike that found in those of the leaf. The chloroplasts in the epidermis, the smallest of those in the tuber, have a diameter from two to three times as great as those in the leaf. Larger chloroplasts, in some few of the superficial cells, depart from the typical lens shape. Their flat surfaces are approximately triangular (Pl. XXV, figs. 26, 28). Their contained starch, in all instances noted, is held within the central vacuole. When these small starch inclusions are examined in polarized light, the two black interference lines can be seen to cross at the center of the grain. Starch in more and thicker layers is deposited on one surface of each of these grains than on the other surfaces and, as a consequence of this mode of growth, the grain is forced to one side of the plastid and forms a projection from one side of it (figs. 27, 28). Whether the starch grain ever actually pushes through the stroma so that parts of it lie in direct contact with the cytoplasm itself, the present investigation did not discover. The chlorophyll-bearing stroma does become localized on the base of the starch grain, and it is on this part of the grain in intimate contact with the mass of the chloroplast that the lamellae are laid down. Thus the hilum is at one extremity of the grain, the mass of the chlorophyll-bearing stroma at the other. No membrane-like stroma can be observed to surround these larger starch grains, yet there is some evidence that the stroma extends farther about the starch than does the pigment-bearing ground substance. When a starch grain is examined in polarized light (Pl. XXVI, fig. 33), it is obvious which portion of it lay next to the mass of the plastid. It frequently happens that a starch grain, which has been developing perfectly regularly by many successive layers, may have

other additional lamellae deposited upon it running at right angles to the earlier ones (fig. 33). In every instance observed, the green portion of the chloroplast lay upon the outermost of these outer lamellae, and the evidence seems to be quite conclusive that the stroma has been shifted about the starch grain. If the starch grain were included in great part in a sort of plastid sac and had become twisted within the sac, we should not have to picture this change in lamellation as being due to the chloroplast's sticking to and creeping about the surface of the starch grain. In either instance, whether the grain is surrounded by the plastid or not, it is the everted *internal* surface of the chloroplast and not the external one which is in contact with the grain. The observed slowing of the photosynthetic process after it has been progressing for a time can not be explained on the ground that the accumulated starch has covered a portion of the surface of the chloroplast and hence reduced its activity.

The cell sap within the Phajus tuber has an osmotic value about the same as that in the leaf. Consequently there is the same swelling of the tuber chloroplasts when they are extruded into water as occurs when the leaf chloroplasts are extruded, though the larger size of the tuber chloroplasts makes their swelling more conspicuous. At times they fragment just as the leaf chloroplasts do (Pl. XXV, figs. 30, 31). Frequently, however, the stroma swells but little and the contents of the central vacuole splits the stroma and exudes, surrounded by a visible surface film (fig. 32). If sugar is now added to the water in which the plastids lie, the expanded vacuolar contents can be made to contract (fig. 29). When thus concentrated, they often have the form and apparent consistency of a pseudopodium of amoeba and could easily be mistaken to be an essential part of the chloroplast ("perispermium") and not an artifact. It is interesting to note that this splitting of the chloroplast occurs regularly in a definite region of the plastid. As can be seen from figure 28, the slits occur in the stroma at the maximum distance from the starch inclusions, which fact would indicate that the chloroplasts are tougher in that region which is stretched out over the starch grain. These slits have also been observed in chloroplasts which had large, bulging starch grains.

#### CHARA

The resemblance of the chloroplasts of the Charales to those of higher plants has long been noted. Indeed, Tschirch (91) drew certain general conclusions concerning the structure of the chloroplasts of higher plants from his investigation of those of *Nitella*. He reported that when two chloroplasts of this alga were pressed together the green portions were always separated by a colorless zone of uniform width, even when the chloroplasts were flattened into polyhedral forms. From this he assumed, as has been stated, that the plastids were surrounded by a membrane. The observations of Tschirch are substantiated in part by the present investigation. The colored portions of two chloroplasts were never observed to come into

actual contact, no matter how closely they were crowded together. The hyaline zone which separates them did not, it is true, have the uniform width of a membrane. The chloroplasts often assumed polyhedral forms when they were well separated, and consequently this form is not necessarily a result of pressure (Pl. XXVII, fig. 48).

It can readily be demonstrated that the chloroplasts of *Chara* are imbedded in non-granular cytoplasm. This cytoplasm is localized about the periphery of the cells and does not participate in the general cytoplasmic streaming or cyclosis. The granules of the circulating cytoplasm were never observed to come into contact with the chloroplasts, and when the granules were carried along near the cell periphery they flowed in "channels" between the chloroplasts. Such a stream of granules would often wind around the chloroplasts but would never approach nearer than 1 micron to their surfaces. The non-granular cytoplasm in which the chloroplasts are imbedded is viscous enough to hold the chloroplasts firm despite the friction of the flowing granular cytoplasm. When the cell is immersed in glycerin, this non-granular cytoplasm coagulates into a gel firm enough to hold large groups of the chloroplasts together when the cytoplasm is torn out of the cell wall by needles. These groups of chloroplasts become imbedded in flat plates of cytoplasm and can then be more easily photographed (Pl. XXVI, figs. 35, 37, 40) than they could be within the cell. The granular cytoplasm disintegrates when extruded into glycerin.

The younger and smaller chloroplasts of *Chara* are much like those of the higher plants; they are, however, somewhat longer in proportion to their width and not so flat, but their general structure is the same. Each contains a large central vacuole with similar pores leading to it from the outside. These pores are smaller than the corresponding ones in the chloroplasts of *Elodea*, and consequently the stroma seems less granular. At times no pores whatever can be observed and the ground substance seems to be homogeneous. As the plastids of *Chara* age and increase in size, especially as starch inclusions are deposited in them, they lose their lens-like form. Though the chloroplasts of *Chara* are thus built on the same general plan as those of the higher plants, they nevertheless form a clearly distinguishable type.

One of the most striking characteristics of these chloroplasts of *Chara* is the variety of shapes among the plastids of a single cell. Certain plastids are nearly spherical while others may be six or seven times as long as wide and have protrusions over certain included starch grains (Pl. XXVII, fig. 48). As a rule, the spherical ones occur next to the neutral zone, while the long and narrow ones are found where cyclosis is most rapid. A polyhedral form (fig. 49) is not at all uncommon.

The chloroplasts in the older part of *Chara*, as was noted by Pringsheim (58), are as a rule larger than those in the younger part. This increase in size is due almost entirely to increased starch content. Chloroplasts within

the same cell, no matter how they may vary in shape, contain approximately the same amount of starch although the included starch in chloroplasts in adjoining cells may differ greatly. The chloroplasts in the newer cells measure on an average 7 by  $5\mu$ . In the older cells of *Chara* the chloroplasts may be 24 by  $14\mu$ , which makes their volume approximately twenty times that of the smaller ones, and they often contain only a faint trace of chlorophyll. Such an increase in size is quite unlike anything observed in the higher plants.

It is in the number and arrangement of the included starch grains that the chloroplasts of *Chara* differ most from the typical lens-shaped ones. While the chloroplasts of the higher plants as a rule have but one grain each, two or three may sometimes be found; those of *Chara* have from five to ten grains each (figs. 45-49). The starch grains in *Chara* are as a rule in the form of prolate spheroids (figs. 35, 40, Pl. XXVI; fig. 45, Pl. XXVII), though when the plants have been kept in the dark for a while they may be spherical (figs. 47, 48). In the older plastids the starch grains are crowded closely together and consequently become polyhedral (Pl. XXVI, fig. 40; Pl. XXVII, fig. 49). The starch inclusions of a chloroplast always occur within a single central vacuole which often becomes so large, relatively, that the enclosing stroma appears as little more than a membrane. The appearance of such chloroplasts makes it easy for us to understand why the earlier workers looked upon them as vesicles. In those chloroplasts which are long and narrow the starch grains occur in rows (fig. 48); an arrangement unlike any that has been observed in the higher plants.

If the resemblance in forms of the chloroplasts is an indication of relationship, it would seem that Charales are not closely related to the liverworts, and so to the mosses or ferns, as some investigators have suggested, but form a very aberrant group of plants.

#### VAUCHERIA

The small, lens-shaped chloroplasts of *Vaucheria* are at first sight quite like the chloroplasts of the higher plants. They have the same size and shape, are in a like manner imbedded in non-granular cytoplasm, and they are arranged about the periphery of the cell. This resemblance, however, is entirely a superficial one, since they contain no trace of included starch and have no vacuoles of any kind but appear to be completely homogeneous. When examined in light of 550 Ångström units, which optically removes all the chlorophyll, no structure whatever can be observed in the stroma. When they are fixed and the chlorophyll is removed by solvents, the stroma appears finely granular, this granulation being probably due to the treatment.

The cytoplasm of *Vaucheria* also contains minute oil droplets which in healthy cells range in size from a diameter of one fourth to one of three times that of the chloroplasts. These droplets have frequently been reported as green in color and as being, in all probability, extrusions into the cell of the

lipoid-chlorophyll solution which is normally contained in the chloroplast. In every healthy cell examined during the present investigation, these droplets of oil have been colorless and a spectrum analysis has shown that they contain no trace of chlorophyll. The presence of these droplets within the cell made possible a suggestive experiment, the results of which indicate that chlorophyll is not contained in the plastid, of *Vaucheria* at least, in a lipoid solution. If *Vaucheria* is immersed in 50% acetone, 50% alcoholic solution, or in boiling water, the chlorophyll is transferred almost instantaneously from the plastids to these oil droplets. The change is quick and spectacular. The oil droplets take the chlorophyll out of the acetone-water or alcohol-water solvent almost as quickly as these solvents remove it from the plastids. The acetone or alcohol in question is not itself colored. The color seems to fade out of the plastid and to appear instead in the oil droplet. However, if solutions are used which contain as much as 80% acetone or alcohol, the oil droplets will themselves be dissolved and the solution will appear green. When the oil droplets come into contact with 60% acetone, they become brilliantly green and swell slightly from the acetone they take out of the acetone-water solution. This transfer of color occurs also in 30% acetone, but in 10% acetone the oil droplets become green only after about twelve hours and even then not all the pigment is taken out of the plastid. The droplets, when fully colored by the pigment, are of a brilliant blue-green, becoming much darker than were the living chloroplasts themselves. An analysis of the spectrum of these droplets gives somewhat uncertain results because of the density of the contained solution. The indications are that they contain both chlorophyll *a* and chlorophyll *b*. The yellow pigments are also transferred to the oil droplets, but their transfer is decidedly slower than that of the green. Upon saponification of their dissolved pigments the droplets become yellow, which is the color of saponified chlorophyll *a*, but the yellow color is not pure, and it is probable that a certain amount of chlorophyll *b* is also present.

The fact that this transfer of color takes place shows definitely that the plastids do not hold the pigments as firmly as do the oil droplets. (Whether or not the pigments are altered by the acetone is irrelevant to any conclusions we may draw from this experiment as to the existence of a chlorophyll-lipoid solution in the plastid.) From this we might conclude either (1) that the chlorophyll exists in the plastid in a lipoid solution dispersed in minute droplets, or (2) that it exists there in some state in which it is not as firmly held as it would be in a lipoid solution. Let us assume the former of these possibilities to be true. Chlorophyll is more soluble in a lipoid than it is in a weak acetone solution, as is shown by the fact that the oil droplets take up the color. Now, if chlorophyll existed in a lipoid solution within the plastid, the acetone-water solution would have to dissolve out the lipoid as well as the chlorophyll, for it can not take chlorophyll out of the lipoid. The acetone-water solution within the cell doubtless contains

a certain amount of lipid in solution; how much is unknown, but, whatever the quantity, the solution is saturated, for the oil droplets do not dissolve. According to our assumption we have an emulsion whose discontinuous phase consists of a lipid in very fine droplets in the plastids and larger oil droplets free in the cell; and a continuous phase consisting of an acetone-water solution which is saturated with lipid. Under these conditions, at the interphase there is a continuous balanced interchange of lipid molecules, as many entering the lipid droplets as leave them. The smaller lipid droplets have, of course, a greater curvature which increases their internal pressure. This pressure increases their solubility and consequently they would tend to dissolve in the acetone-water solution and be taken out of this solution by the oil droplets within the cell. This would account for the transfer of color to the oil droplets.

An objection which practically invalidates this hypothesis is that it would require some time for the emulsified lipid to collect in the larger droplets. But in fact the coloring of the oil droplets is practically instantaneous. Even if the hypothetical lipid in which the chlorophyll is assumed to be dissolved were much more soluble in acetone than the oil droplets were, consequently would dissolve more quickly than a mere consideration of the size of the droplets would lead us to expect, the transfer of color would hardly be instantaneous through any medium which was 70% water.

It seems more reasonable to suppose that the chlorophyll in the plastid is held in some state in which it can be more readily extracted by a water-acetone solution than it could be if it were dissolved in a lipid. This latter hypothesis is in harmony with the inferences drawn from the photostability of chlorophyll in dried plastids and in plastids immersed in glycerin.

#### SUMMARY

1. The ground substance or stroma of the chloroplast is in the form of a hollow, flattened, prolate spheroid surrounding a large central "vacuole." The granular appearance of this stroma is due to numerous pores which connect the central vacuole with the cytoplasm surrounding the chloroplast.

2. The chloroplast is surrounded by a more or less permanent sheath of non-granular cytoplasm.

3. No evidence was found of the existence of an osmotic membrane around the chloroplast.

4. Certain cytoplasmic granules are quite refractive when they have a chloroplast for a background and are practically invisible in white light. They therefore appear to be part of the chloroplast. In monochromatic light of 550 Ångström units it can be seen, however, that they do not really belong to the chloroplast.

5. The pigments of the chloroplast are intimately mixed and evenly distributed throughout the protein ground substance.

6. In the leaf tissue the starch inclusions of the chloroplast are contained within the central vacuole.



7. In certain storage tissues, a large starch grain may appear to be attached to the surface of a chloroplast. This grain has merely bulged out from the central vacuole and is in contact with the everted inner surface of the hollow stroma, not with the *external* surface.

8. The frequently observed swelling of chloroplasts when extruded into water is believed to be due to the imbibition of water by the contents of the central vacuole which remains separated from the outer water by a surface film. These contents could not be stained and are believed to consist mainly of water with a little sugar and protein in solution.

9. Little difference could be observed between the chloroplasts of distantly related seed plants which are placed quite far apart in the systematic scheme.

10. In many leaves a constant differentiation of the chloroplasts is observable. Certain plastids contain little starch and are mainly concerned with carrying on photosynthesis. Others contain much included starch and little green tissue and seem to function mainly as storage organs.

11. The photostability of chlorophyll in a plastid extruded from the cell would indicate that the chlorophyll is not in a lipid solution, for chlorophyll in solution is quickly destroyed by light. Chlorophyll can also be extracted from chloroplasts by solvents which can not extract it from a lipid solution.

12. Green tissue in 100% glycerin will not be bleached by direct sunlight even after several months' exposure.

13. The staining properties and reactions to enzymes of bleached and unbleached chloroplasts indicate that the chlorophyll coats the colloidal particles of the stroma.

14. The chloroplasts of *Chara* and *Vaucheria* are only superficially like those of the higher plants.

The author wishes to acknowledge his indebtedness to Prof. R. Ruggles Gates of the University of London and to Prof. Robert Chodat of the University of Geneva for their valuable assistance and their courtesy in extending to him the use of their laboratories; and to Prof. W. A. Patrick and Prof. B. E. Livingston of the Johns Hopkins University for their most valuable help in the chemical and physiological aspects of the problem. His thanks are especially due to Prof. Duncan S. Johnson of the Johns Hopkins University, at whose suggestion the present work was undertaken and whose interest and continued assistance made its completion possible.

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## EXPLANATION OF PLATES

### PLATE XXIII

FIGS. 10, 11, 12. Chloroplasts from different regions of the same *Elodea* leaf stained with iodine. The differences in size of the chloroplasts are due to the starch inclusions.  $\times 480$ .

FIG. 13. Chloroplasts of *Elodea* after a 4-minute treatment with ethyl alcohol. The pigment has collected in droplets upon the surface of the chloroplasts and probably consists mainly of ethyl-chlorophyllide dissolved in phytol.

FIG. 14. Chloroplasts of a fern prothallium after being immersed 30 seconds in boiling water. The expanding starch inclusions have left the central vacuoles clearly visible.

FIG. 15. Chloroplasts from first leaf of young fern sporophyte (boiled 30 seconds).

FIG. 16. Chloroplasts from the prothallium attached to the sporophyte shown in figure 15 (boiled 30 seconds).

### PLATE XXIV

FIG. 17. Chloroplasts of a fern prothallium. Water was admitted to the ruptured cell, with the result that the substances within the central vacuoles of the chloroplasts became swollen. The granular stroma can be seen at one side of the swollen vacuole. The membrane surrounding the vacuole is merely the surface film of the vacuole contents in contact with water.

FIG. 18. Chloroplasts of *Cabomba*. The excentric starch inclusions are always on that side of the chloroplast nearer the center of the cell.

FIG. 19. Chloroplasts of a leaf of *Phajus*.

FIG. 20. Chloroplasts of *Conocephalum*. Fixed in picric acid, sectioned in paraffin, and stained with iron-alum haematoxylin.  $\times 480$ .

FIG. 21. Chloroplasts of a leaf of *Phajus* stained with iodine to show starch inclusions.

FIG. 22. Chloroplasts of leaf of *Phajus* in contact with water. The substances of the central vacuole are swollen.

FIG. 23. Fragments of chloroplasts of *Pallavicinia* boiled 30 seconds. The expanding starch inclusions have exploded the chloroplasts.

FIG. 24. Same as figure 22. The stroma can be seen as fragments upon the surface of the swollen central vacuole.

### PLATE XXV

FIG. 25. Chloroplasts of *Ceratophyllum*. Fixed by boiling 30 seconds.

FIG. 26. Chloroplasts of the tuber of *Phajus*, grouped about the nucleus. Compare the size of these chloroplasts with those of the leaf shown in figure 19.

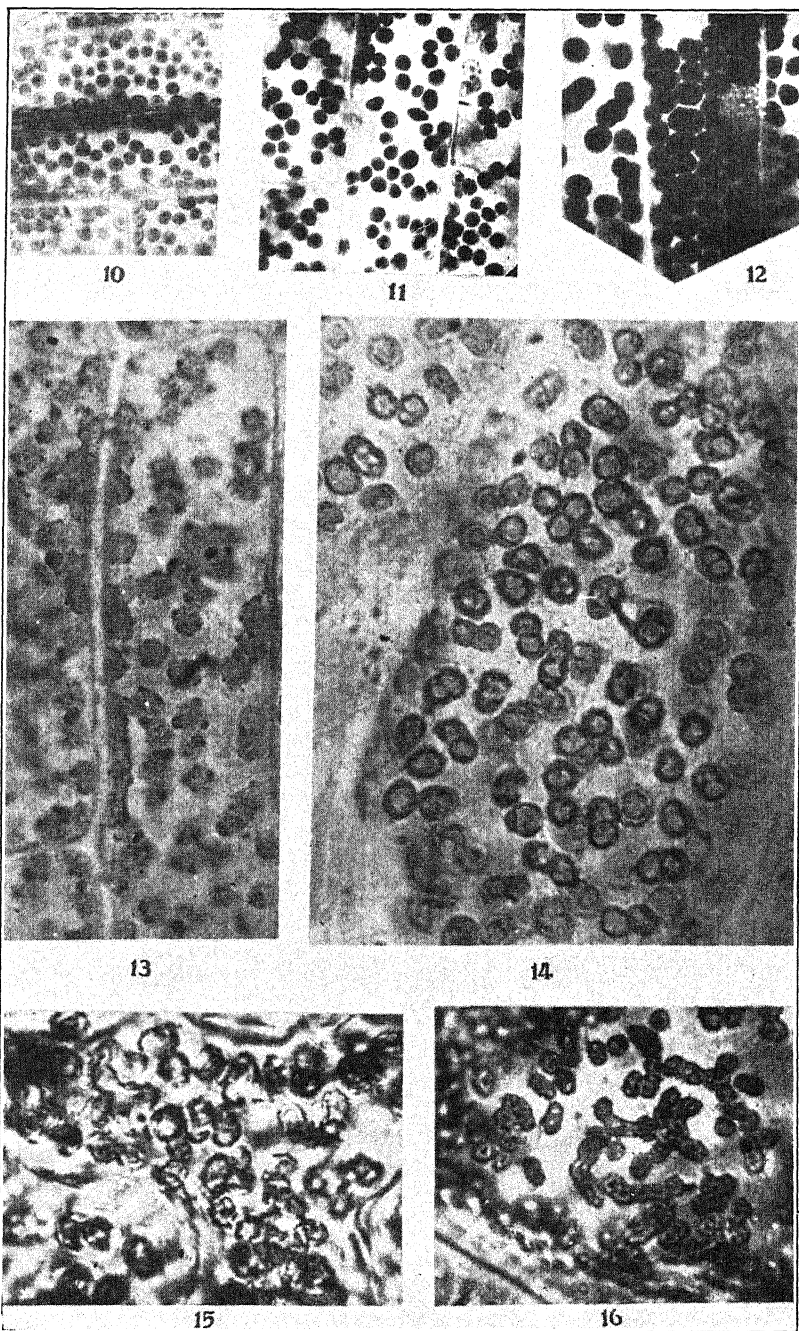
FIG. 27. Chloroplasts in tuber of *Phajus* showing the excentric position of starch inclusion. Compare with figure 21.  $\times 220$ .

FIG. 28. Chloroplasts in tuber of *Phajus*, each showing a starch inclusion and a slit. The slit was caused by the expanding contents of the central vacuole. The contents of the vacuole exuded through the slit and finally dissolved in the surrounding water.

FIG. 29. Chloroplast in the tuber of *Phajus*, treated as those shown in figure 28 except that the swelling of the contents of the vacuole was halted with cane sugar. The contents of the central vacuole can be seen extruding from both ends of the chloroplast.

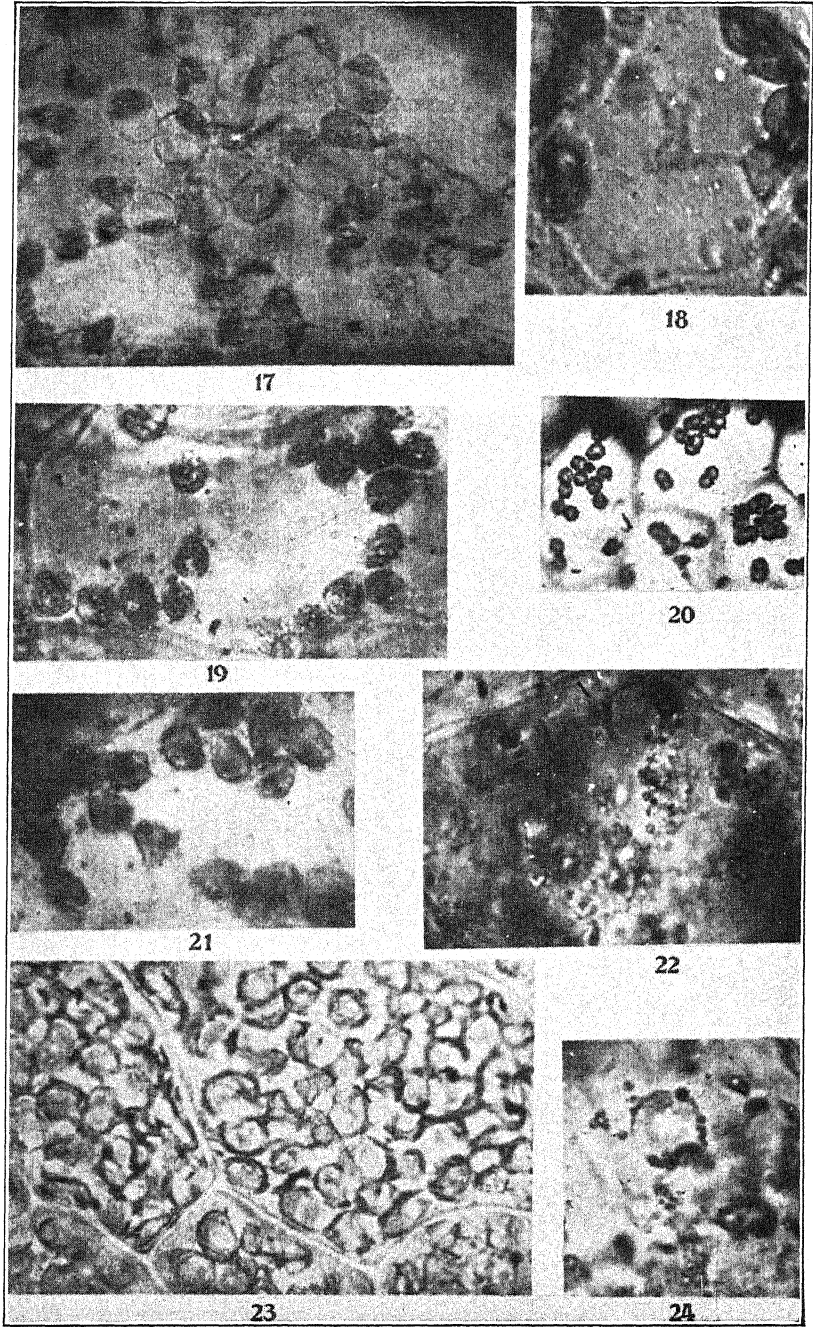
FIG. 30. Chloroplast from tuber of *Phajus* extruded into tap water. The fragments of the stroma can be seen upon the surface of the swollen vacuolar contents. Compare the size with that of the chloroplast shown in figure 24.

FIG. 31. Same as figure 30.  $\times 480$ .



ZIRKLE: STRUCTURE OF CHLOROPLAST

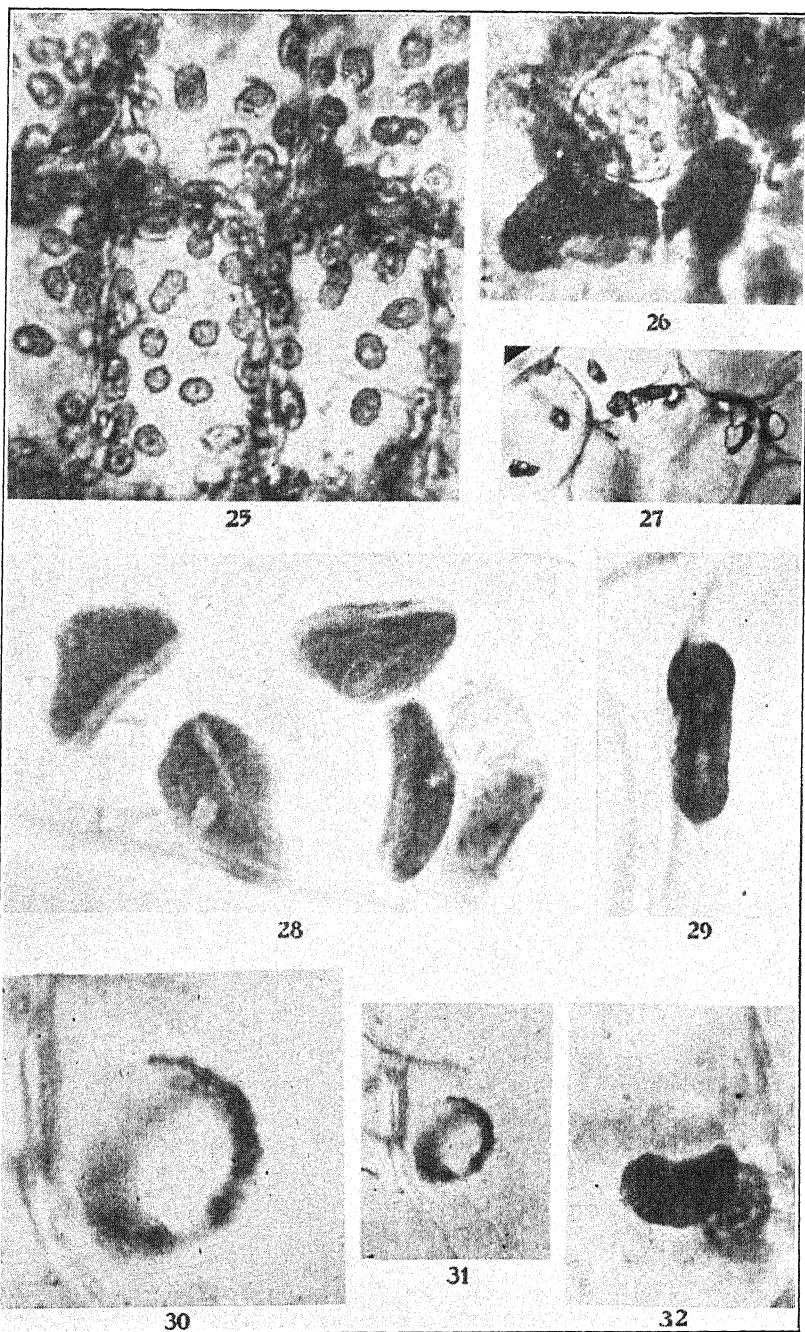




ZIRKLE: STRUCTURE OF CHLOROPLAST

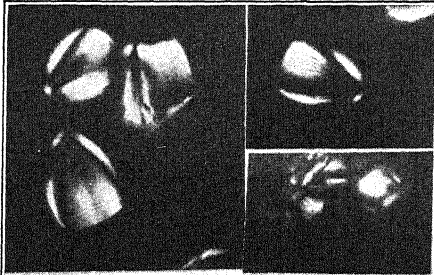




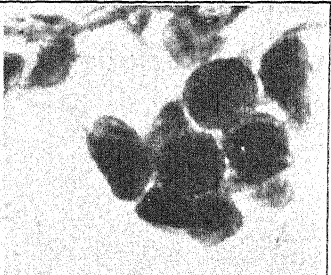


ZIRKLE: STRUCTURE OF CHLOROPLAST

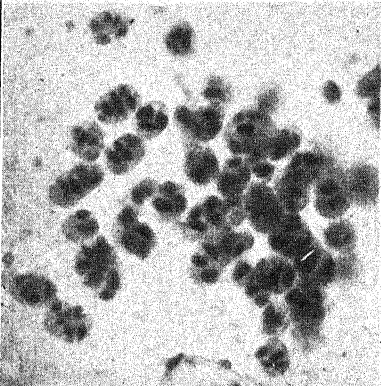




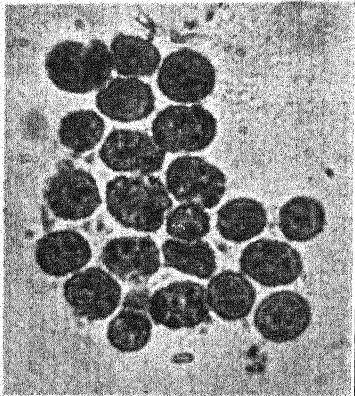
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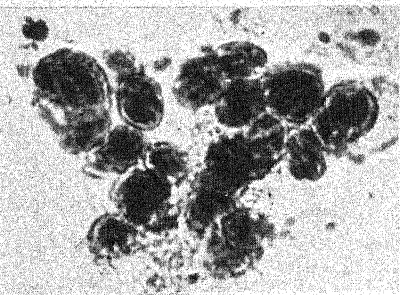
34



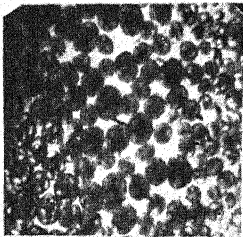
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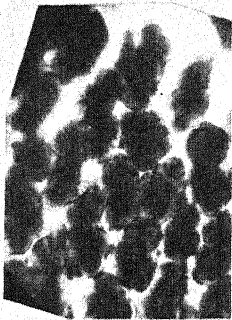
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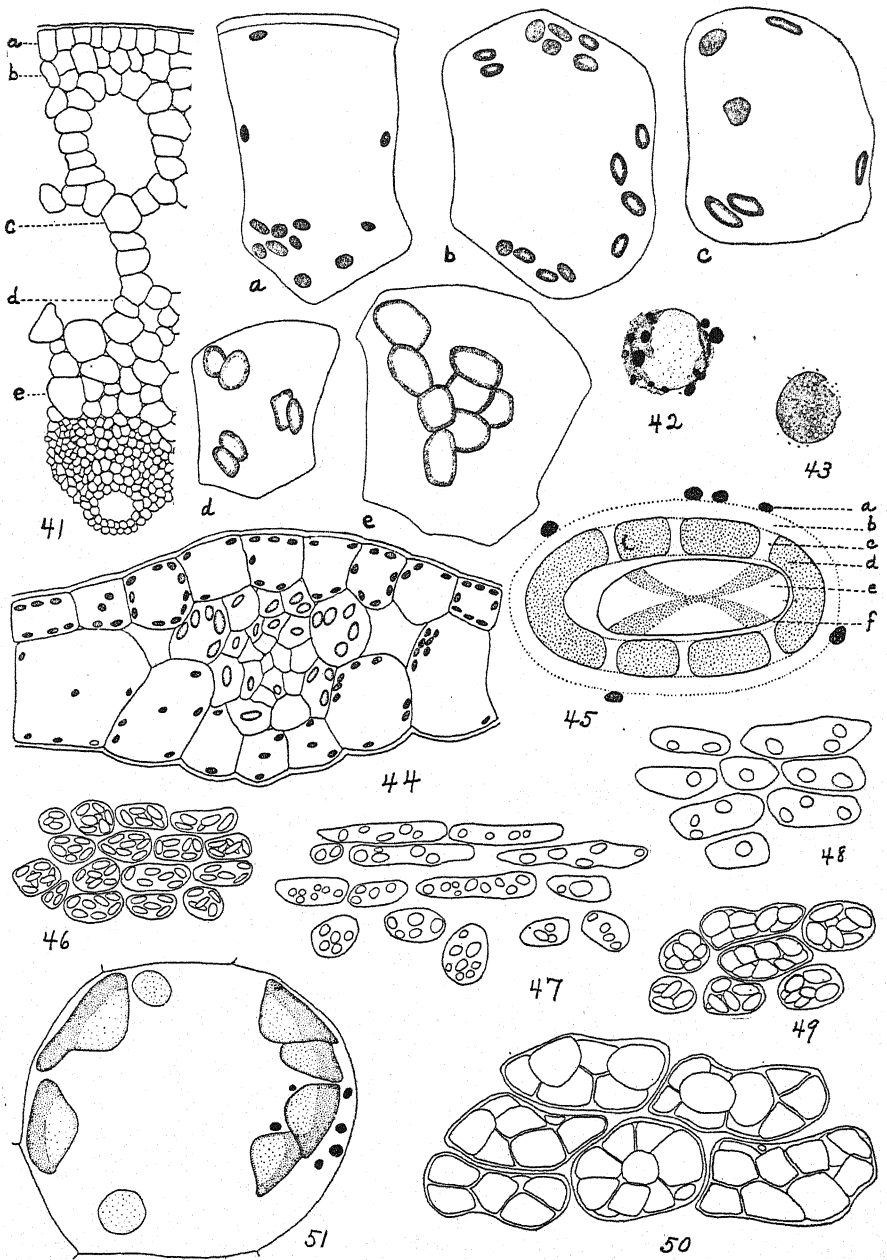


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ZIRKLE: STRUCTURE OF CHLOROPLAST



FIG. 32. Chloroplast of tuber of *Phajus* treated as were those shown in figure 28. The slit is in profile and does not show. The contents of the central vacuole can be seen exuding from the chloroplast. It is separated from the surrounding water by an interphase membrane.

## PLATE XXVI

FIG. 33. Starch grains from tuber of *Phajus* photographed in polarized light.  $\times 170$ .

FIG. 34. Chloroplasts from leaf of *Peperomia hispidula* stained with iron-alum haematoxylin. Two kinds of chloroplasts can be seen in the adjoining cells of the leaf.

FIGS. 35, 36, 37. Chloroplasts of *Chara* extruded into glycerin, stained with iodine to show starch inclusions.

FIG. 38. Chloroplasts in tuber of *Phajus* showing attached starch grains.  $\times 220$ .

FIG. 39. Chloroplasts of *Chara* showing starch inclusions.  $\times 480$ .

FIG. 40. Chloroplasts of *Chara* stained with iodine to show starch inclusions.  $\times 480$ .

## PLATE XXVII

FIG. 41. Diagram of segment of a transverse section of a stem of *Elodea*.  $\times 90$ . *A*, *B*, *C*, *D*, and *E* are cells in the transverse section located as indicated, drawn to a larger scale.  $\times 750$ . These cells contain diagrams of the various types of chloroplasts which occur in different regions in the stem.

FIG. 42. Swollen chloroplast of *Vallisneria* extruded into water.  $\times 900$ .

FIG. 43. Slab-section of a chloroplast of *Peperomia*, showing pores through the stroma.  $\times 670$ .

FIG. 44. Transverse section of midrib in leaf of *Elodea* showing location of specialized starch-storing chloroplasts.  $\times 575$ .

FIG. 45. Idealized diagram of a cross section of the type chloroplast of the higher plants: *a*, cytoplasmic granule adhering to the cytoplasmic sheath; *b*, cytoplasmic sheath; *c*, pore in stroma; *d*, stroma; *e*, starch inclusion as it appears in polarized light; *f*, central vacuole.

FIGS. 46-50. Chloroplasts of *Chara* with outlined starch inclusions. Figures 46, 49, and 50 were drawn from a plant which had been growing in direct sunlight. Figures 47 and 48 were taken from a plant which had been kept a month in the diffused light of the laboratory. The rounded chloroplasts in figure 47 are next to the neutral zone.  $\times 750$ .

FIG. 51. Diagram of cell of *Cabomba* showing location of starch inclusions in the chloroplasts.  $\times 900$ .



# INTERNAL DECLINE (ENDOXEROSIS<sup>1</sup>) OF LEMONS IV. THE CARBOHYDRATES IN THE PEEL OF HEALTHY AND ENDOXEROTIC FRUITS<sup>2</sup>

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## INTRODUCTION

Endoxerosis is a physiological disease of lemon fruits, mainly characterized by a loss of water from the internal tissues (4, 5). In a very large percentage of instances one of the first visible internal evidences of injury is the presence of gum in the conducting vessels and surrounding tissues, especially in the peel (3). Spoehr (16), Hooker (11), Rosa (14), and others have shown that the amounts of pentoses and pentosans in certain plants may be greatly influenced by temperature and by the water content of the tissues. Spoehr concluded that continued low water content in the tissues and high temperature are conducive to the transformation of monosaccharids into pentosans. Since these conditions appear to be most favorable for the production of endoxerosis in lemons, and since gum is often manifestly present in parts of the affected tissues, it seemed desirable to make comparative quantitative determinations of the carbohydrates, especially the pentoses and pentosans, in healthy and endoxerotic lemons.

## MATERIALS

The tissues used in the tests were taken from the peels of nearly mature lemons. A thin outer layer was cut from about one fourth of the stylar end of each lemon to remove most of the portion of the peel containing the coloring matter and the oil glands. The remaining albedo or white portion of the peel, which contains the tissues first affected by endoxerosis, was then cut off and used for making the tests. The tissues were ground in a meat chopper and lots 15*a* and *b*, 17*a* and *b*, and 19*b* and *c* were then placed in covered vessels and exposed to a temperature of 100° C. for one hour, after which they were dried in a ventilated oven at 60° or 65° C. (the "*b*" in each lot indicates that the tissues were taken from healthy lemons and the "*a*" or "*c*" that they were taken from endoxerotic lemons.) They were then powdered to pass through a 100-mesh sieve. Lots 18*a* and *b* were not

<sup>1</sup> The term endoxerosis, as previously suggested (5), is a technical name for this malady, and while it is so used in the text of this paper the term *internal decline* is retained in the title to facilitate references to this series of papers.

<sup>2</sup> Paper no. 131, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California. The analyses were made by the junior author.

dried but were placed at once in 95% alcohol where they remained until analyzed.

The more or less pure citrus gum used had exuded from a lemon tree affected with gummosis. The dry, hard, light-amber pieces of gum were pulverized to pass a 100-mesh sieve.

#### METHODS

The methods used in extracting the carbohydrates of the albedo of the lemon peel were essentially those used by Spoehr (16) in his study of the carbohydrate economy of the cacti. The dry, powdered material was heated for 3 hours on a water bath under a reflux condenser with 1% HCl. Forty cc. of the 1% HCl were used for every gram of air-dry material. Determinations were also made using 0.135% HCl instead of the 1% HCl. The extract was filtered and the residue thoroughly washed with hot water, the washings being added to the filtrate. The filtrate was made neutral to methyl red with NaOH<sup>3</sup> and made up to definite volume. This solution was called *A*, and its reducing value was determined. An aliquot of *A* was fermented<sup>4</sup> by yeast, as described later, filtered, evaporated on a water bath to two thirds of its volume, and made up to definite volume. This solution was called *B*, and its reducing value was determined.

A second sample of the dry, powdered material was heated on a water bath under a reflux condenser for 3 hours with 95% alcohol and powdered calcium carbonate. Ten cc. of alcohol and 0.06 g. of calcium carbonate were used for every gram of dry material. The alcohol was filtered off, and the residue was boiled with a fresh lot of alcohol for 3 hours. The second lot of alcohol was filtered off and added to the first. The residue was washed thoroughly with alcohol and the washings were added to the filtrates. The yellowish alcoholic solution was evaporated to dryness on a water bath, dissolved in water, and made up to definite volume. This solution was called *C*, and its reducing value was determined. To an aliquot of *C*, 5 cc. of *N*/1 HCl were added for every 100 cc. taken, and the acidified solution was heated on a water bath for 3 hours, the water lost by evaporation being replaced from time to time. The acid was made neutral to methyl red with NaOH and made up to definite volume. This solution was called *D*, and its reducing value was determined. An aliquot of *D* was then fermented by yeast as described later, filtered, reduced, by heating on a water bath, to about two thirds of its volume, and then made up to definite volume. This solution was called *E*, and its reducing value was determined.

The dry weight of a third sample was determined by heating at 100°C.

<sup>3</sup> NaOH was preferred to Na<sub>2</sub>CO<sub>3</sub> for neutralization because the CO<sub>2</sub> lost in boiling by a solution neutralized with Na<sub>2</sub>CO<sub>3</sub> was found in some cases to make the solution alkaline enough to destroy pentoses.

<sup>4</sup> The term "fermented" as used in this paper means to change to other substances by any process of metabolism.

### Determination of Reducing Value of Solutions

The reducing value of the solutions was determined by heating with Fehling's solution, centrifuging, and titrating the copper sulfate left in an aliquot of the supernatant liquid with *N*/20 sodium thiosulfate. This method has been thoroughly tested and described by Peters (12, 13). It has also been used and discussed by Spoehr (16).

### Calculation of Results

The various carbohydrates present were calculated from the reducing values of the five solutions, designated as *A*, *B*, *C*, *D*, and *E*. There is no starch in the peel of the lemon.

According to Spoehr, if the reducing value of dextrose is taken as 1, the reducing value of invert sugar is 0.95, of pentose (xylose) 0.90, of hexose polysaccharids 0.90, and of pentosan 0.85. According to Peters the dextrose-copper factor is 0.522. Therefore the sugar value of a given solution = mg. copper reduced  $\times 0.522 \times$  the suitable factor for the particular carbohydrate.

The hexoses =  $C - E$ .

Disaccharids =  $D - C$ .

Hexose polysaccharids (hexosans) =  $(A - B) - (D - E)$ .

Pentoses =  $E$ .

Pentosans =  $B - E$ .

### Determination of Pentoses and Pentosans<sup>5</sup>

Pentoses and pentosans were determined by the fermentation method used by Davis and Sawyer (10), Spoehr (16), Rosa (14), and others. This is based upon the supposed inability of yeast to ferment pentoses. Pentoses were determined by fermenting with yeast the sugar soluble in 95% alcohol and determining the reducing value of the residue after removing, by boiling, the products of fermentation. The pentosans were determined as pentoses after fermenting a 1% HCl extract of the plant material. The reducing value of the fermented solution is supposed to represent the pentoses originally present and those produced by the hydrolysis of the pentosans.

In the earlier stages of the investigation described in this paper Fleischmann's yeast was used for fermenting the sugars. To 50 cc. of the solution made neutral to methyl red, traces of  $\text{NH}_4\text{NO}_3$  and  $\text{KH}_2\text{PO}_4$  were added. A quantity, approximately twice the size of a pea, of Fleischmann's yeast which had been thoroughly washed on a Buchner funnel was suspended in this solution. The mixture was incubated for 17 to 44 hours at 30° C. At the end of the fermentation period, the solution was filtered to remove as

<sup>5</sup> Later discussion will indicate that there was reason to question the purity of these substances as determined, and for this reason it is desired that they should be considered as *apparent* pentoses and pentosans, even though the word "apparent" is omitted in the tables and, except in one paragraph, in the text.

much of the yeast as possible and the filtrate was evaporated on a water bath to about two thirds of its volume. It was then made up to volume, and its reducing value was determined. Rosa cleared the fermented solution, after 10 minutes of boiling, with lead acetate before determining the reducing value. Spoehr evaporated the fermented solution under reduced pressure but did not clear with lead acetate. In the present tests it was found by experimentation that boiling the fermented solution on a water bath did not destroy added xylose, provided the solution was slightly acid to litmus. If, however, it was slightly alkaline the added xylose was partially destroyed by boiling the solution on a water bath. Whether reducing materials were present that were not driven off by boiling and that might have been removed by lead acetate was not determined.

Using the method as described, it was found that the extracts treated with the washed Fleischmann's yeast frequently showed a heavy film of bacteria and mycoderma on the surface by the end of the fermentation period. Successive determinations on the same extract showed considerable variation in the apparent pentose value. For this reason the method was modified by adding 8% of 95% alcohol (by volume) to the solution, before fermentation began. It was thought that this would prevent the development of foreign organisms and still permit the yeast to ferment the hexoses present. It was found that the addition of the alcohol prevented the development of the surface film and apparently, judging from the appearance of the solutions, eliminated foreign organisms. The values secured by fermenting in the presence of the added alcohol were generally higher than those secured without the addition of the alcohol and were more uniform in successive determinations on the same extract. Nevertheless, if simultaneous fermentations were carried out on a given extract, and on the same extract to which xylose or glucose had been added, the xylose could not always be recovered and the solutions to which the glucose had been added generally showed a lower value than the check.

Pure cultures of yeast were isolated from a cake of Fleischmann's yeast and grown in quantity in a solution made by adding sugar and mineral salts to the water extract of dried peaches. This pure yeast was filtered off, washed, and used with the alcohol and mineral-salt addition in place of the commercial Fleischmann's yeast. The results secured were no more satisfactory than those secured with Fleischmann's yeast plus alcohol. In some cases added xylose could be recovered, in others it could not.

The plant extracts made slightly acid to litmus and with the addition of  $\text{NH}_4\text{NO}_3$  and  $\text{KH}_2\text{PO}_4$  were sterilized by intermittent heating in an Arnold sterilizer, inoculated with pure cultures of yeast isolated from Fleischmann's yeast, and fermented under sterile conditions. Even under such conditions added xylose could not always be recovered.

In the determinations of pentoses and pentosans reported in this paper the solutions were fermented in the presence of the added 8% alcohol with

Fleischmann's yeast or pure yeast, or under sterile conditions by pure cultures of yeast isolated from Fleischmann's yeast. The analytical data given later show that in some cases solutions were fermented without duplicates to which glucose or xylose had been added, and that in other cases in which xylose had been added it was not always wholly recovered.

#### ANALYTICAL DATA

The analytical data secured with samples 15, 17, 18, and 19 are given in table 1. The copper reduced per gram of dry weight of tissue is given for

TABLE 1. *Reducing Power of the Various Extractions from Peel of Healthy and Endoxerotic Lemons, Expressed in Grams of Copper per Gram of Dry Weight of Tissues (Healthy Lemons, in this Table and in Tables 2 and 4, Lettered "b")*

Extraction	Grams Copper per Gram Different Tissues							
	15a	15b	17a	17b	18a	18b	19c	19b
A (0.135% HCl)....	0.5320	0.7076	0.4774	0.5567	0.9699	1.0951	0.9952	0.8386
A (1% HCl).....	1.0890	1.1651	—	—	1.1970	1.2168	1.3067	1.0653
B (0.135% HCl)....	0.1781	0.1613	0.2548	0.2106	0.3396	0.2821	0.2606	0.2406
B (1% HCl).....	0.7456	0.3992	—	—	0.5560	0.3832	0.5342	0.3501
C.....	0.2187	0.4199	0.2150	0.2570	0.4967	0.5336	0.4550	0.2748
D.....	0.3357	0.5510	0.2715	0.2806	0.5158	0.6743	0.5348	0.3852
E.....	0.0528	0.0803	0.0955	0.1273	0.0894	0.0756	0.0756	0.1055

each of the five fractions. Extractions were made from samples 15, 18, and 19 with both 1% and 0.135% HCl. There are therefore two determinations of *B*, one for each of these fractions. Determinations of *C* and *D* for samples 15 and 19 were made in duplicate extractions, and for sample 18 in triplicate extractions.

The determinations of *B* in the 0.135% HCl extract of sample 15 were made by fermenting with washed Fleischmann's yeast in the presence of 8% alcohol at 30° C. for 17 hours. For the 1% HCl extract, sterile yeast for five days at 30° C. was used. In duplicate cultures to which 0.025 g. of xylose was added, none was recovered for 15a, and 0.0148 g. was recovered for 15b. *E* was determined by fermenting the hydrolyzed alcoholic extract under sterile conditions for five days at 30° C. Glucose added to duplicates was completely fermented. Of 0.025 g. of added xylose, 0.0213 g. was recovered for 15a, and 0.0207 g. was recovered for 15b.

For sample 17, *B* was determined by fermenting in the presence of 8% alcohol with washed Fleischmann's yeast for 17½ hours. *E* was determined by fermenting with washed Fleischmann's yeast for 40 hours at 30° C. (In consequence of an error, 14% alcohol was added instead of 8%, and the determinations were therefore probably too high.)

For sample 18, *B* was determined in the 0.135% HCl extract by fermenting in the presence of 8% alcohol with washed Fleischmann's yeast for 18 hours at 30° C. In the 1% HCl extract, *B* was determined by fermenting

with sterile yeast for 5 days. Added glucose was fermented, and of 0.025 g. of added xylose none was recovered. The reducing material found after 46 hours' fermentation with pure yeast in the presence of 8% alcohol, and after 6 days' fermentation with sterile yeast, agreed well with that left after 5 days' fermentation with sterile yeast. *E* was determined by fermenting under sterile conditions for four days at 30° C. Added glucose was entirely fermented. Of 0.025 g. xylose added to each of 18*a* and 18*b*, 0.0237 g. was recovered for 18*a* and 0.0285 g. for 18*b*.

For sample 19, *B* was determined in the 0.135% HCl extract by fermenting with washed Fleischmann's yeast for 20 hours in the presence of 8% alcohol. Added glucose was entirely fermented. Of 0.025 g. of added xylose, 0.0157 g. was recovered for 19*b* and 0.0131 g. was recovered for 19*c*. In the 1% HCl extract, *B* was determined by fermenting under sterile conditions for four days at 30° C. Added glucose was completely fermented. Of 0.025 g. of xylose added, 0.009 g. was recovered in 19*c* and 0.0116 g. in 19*b*. *E* was determined by fermenting under sterile conditions for four days at 30° C. Added glucose was fermented and xylose was recovered.

An examination of table 1 shows that the reducing values for the 1% HCl extract are the more uniform, the greatest variation between samples being 22.5%. In two cases in *A*, the endoxerotic lemons show a lower value for this fraction than the healthy ones, but the average for the former is 4% higher than for the latter. With the 0.135% HCl extract, three of the samples show a lower value for the endoxerotic than for the healthy lemons, but the average for the latter is 8% higher than for the former. In *B* the value for the endoxerotic lemons is higher in every instance than for the healthy ones, and the differences are more marked in the 1% than in the 0.135% HCl extracts. For the *C* fractions the average for the endoxerotic lemons is 7% lower than for the healthy ones, and for the *D* fraction 14% lower. The values for *E* are, in three of the four cases, lower for the endoxerotic lemons than for the healthy ones, the average being 8% lower for the latter than for the former.

In table 2 the quantities of the various carbohydrates, as calculated from the data in table 1, are expressed in percentage of dry weight.

A surprisingly large quantity of carbohydrate material is present in the albedo of the lemon peel, from 50 to 62% of the dry matter being composed of reducing material calculated as carbohydrates soluble in 95% alcohol or hydrolyzable in 1% HCl. That these figures are essentially correct is indicated by the following facts. In sample 18 the material was preserved in 95% alcohol. The dry weight of the material dissolving in the alcohol was 26.8% of the total dry weight of 18*a*, and 32.2% of that of 18*b*. The total alcohol-soluble sugars found in these solutions constituted 26.35% of the dry weight for 18*a* and 34.43% for 18*b*. The polysaccharids hydrolyzed by 1% HCl were approximately constant in amount, making up from 24.68 to 35% of the dry weight, and were from 5 to 17% higher in the endoxerotic

lemons than in the healthy ones. Compared with the polysaccharids, the total mono- and disaccharids show a greater variation, from 13.52 to 34.43% of the dry weight, and they are not constantly greater in either of the two types of samples, *i.e.*, in that from the healthy or in that from the endoxerotic lemons.

TABLE 2. *Carbohydrates in the Peel of Healthy and Endoxerotic Lemons Expressed as Percentage of Dry Weight (Calculated from Data in Table 1)*

Kind of Carbohydrates	Percent Dry Weight of Tissue Sample							
	15a	15b	17a	17b	18a	18b	19c	19b
Hexoses, <i>C - E</i> .....	8.66	17.72	6.24	6.77	21.20	23.91	19.80	8.84
Disaccharids, <i>D - C</i> .....	5.80	6.50	2.80	1.17	0.95	6.97	3.95	5.46
Hexosans, 0.135% HCl, ( <i>A - B</i> )- ( <i>D - E</i> ).....	3.33	3.55	2.19	9.06	5.35	10.07	12.94	15.00
Hexosans, 1% HCl, ( <i>A - B</i> )- ( <i>D - E</i> ).....	2.84	13.87	—	—	5.38	11.03	14.72	20.46
Pentosans, 0.135% HCl, <i>B - E</i> ...	5.12	3.59	7.07	3.69	11.10	9.16	8.65	5.99
Pentosans, 1% HCl, <i>B - E</i> .....	30.74	14.15	—	—	20.68	13.65	20.35	10.85
Pentoses, <i>E</i> .....	2.48	3.77	4.48	5.98	4.20	3.55	3.55	4.96
Total, 0.135% HCl.....	25.39	35.13	22.78	26.67	42.80	53.66	48.89	40.52
Total, 1% HCl.....	50.52	56.01	—	—	52.41	59.11	62.37	50.57
Mono- and disaccharids.....	16.94	27.99	13.52	13.92	26.35	34.43	27.30	19.26
Polysaccharids.....	33.48	28.02	—	—	26.06	24.68	35.07	31.31
Hexosans: Healthy minus endoxerotic.....		11.03				5.65		5.74
Pentosans: Endoxerotic minus healthy.....	16.59				7.03		9.50	

Comparing the effect of 0.135% with that of 1% HCl hydrolysis of the polysaccharids, the increase in pentosans for the endoxerotic lemons was about twice that for the healthy, but there was little increase in the hexosans. With the healthy lemons the 1% HCl extractions increased the values of the hexosans and pentosans about equally. It is rather surprising that there was not a more marked increase in the hexosans in the 1% HCl extractions, since an analysis of the gum from the bark of a lemon tree, affected with gummosis, indicated a 38% hexosan and a 62% pentosan content. Calculating from the data given in table 5, there was in one gram of the gum 0.5925 g. of pentosan and 0.3654 g. of hexosan, making a total of 0.9579 g. We should therefore expect that, if the 1% HCl hydrolyzed more gum in the endoxerotic lemons than was hydrolyzed by the more dilute acid, some of the product would be fermentable and appear as hexosans in table 2. The gum was hydrolyzed with 1% HCl<sup>6</sup> and fermented in the presence of 8% alcohol with washed pure yeast. After 46 hours' fermentation (table 3), the unfermentable residue was constant in amount. It is possible that the

<sup>6</sup> When the gum was hydrolyzed with 0.135% HCl, about 35%, and when hydrolyzed with 0.4% HCl, 72% of the reducing value found with 1% HCl was secured. This result was due to incomplete hydrolysis of the gum with the weaker acids, as some of the unhydrolyzed gum could be precipitated by the addition of alcohol to the extracts made by the two weaker solutions of HCl.

TABLE 3. *Reducing Values for Gum from a Lemon Tree Affected with Gummosis, after Hydrolysis with 1% HCl and Various Periods of Fermentation with Washed Pure Yeast at 30° C.*

Extract and Additions	Time of Fermentation Hours	Copper per G. Dry Wt. of Gum G.
1% HCl extract.....	None	2.1155
1% HCl extract + 8% alcohol.....	19	1.4937
1% HCl extract + 8% alcohol.....	46	1.3262
1% HCl extract + 8% alcohol.....	67	1.3492

gum which exudes from a tree affected with gummosis and dries slowly in the air and sunlight is not identical in composition with the gum present in the endoxerotic lemons.

TABLE 4. *Ratios between Various Carbohydrates in the Peels of Healthy and Endoxerotic Lemons as Extracted by 1% HCl Hydrolysis*

Tissue Sample	Pento- sans	Pento- sans	Pento- sans	Pento- sans	Hexosans	Hexosans	Hexosans	Polysac- charids
	Total Carbo- hydrates	Hexoses	Hex- osans	Hex- osans and Hexoses	Total Carbo- hydrates	Hexoses	Total Carbo- hydrates	Total Carbo- hydrates
15a.....	0.608	3.56	10.8	2.7	0.056	0.33	0.171	0.66
15b.....	0.252	0.79	1.0	0.37	0.247	0.78	0.316	0.50
18a.....	0.394	0.90	3.8	0.83	0.102	0.20	0.404	0.49
18b.....	0.231	0.57	1.2	0.38	0.186	0.46	0.404	0.42
19c.....	0.326	1.03	1.4	0.62	0.236	0.74	0.319	0.56
19b.....	0.214	1.22	0.5	0.37	0.404	0.23	0.174	0.62

In table 4 the calculated ratios between the carbohydrates, as determined by 1% HCl hydrolysis, are indicated, particular attention being paid to the polysaccharids and their relation to the other carbohydrates.

Most noteworthy of the results shown in table 4 is the relative constancy of the pentosan content of the healthy lemon. This is particularly noticeable in the ratio between the pentosans and the total carbohydrates. The polysaccharids in relation to the total carbohydrates are approximately constant in both healthy and endoxerotic lemons. The ratio of hexosans to total carbohydrates is always less in the endoxerotic than in the healthy lemons of the same lot, and the ratio of pentosans to total carbohydrates is always higher in the endoxerotic lemons than in the healthy ones.

#### DISCUSSION

No attempt has been made in this investigation to determine the specific origin of the various carbohydrates mentioned, the attempt has been merely to determine their comparative amounts in healthy and endoxerotic lemons. The determination of the reducing values of the solutions was comparatively simple and accurate, but the isolation of the pentoses by the fermentation method proved more difficult, much more so than was anticipated when the analytical tests were begun.



The investigation was not directed toward the development of methods for determining pentoses, since it was assumed from the work of the more recent previous investigators that the method was dependable. In the hands of Spoehr (16) the fermentation method for the isolation of pentoses gave excellent results, as is evidenced by the fact that he was able to determine the pentoses in artificial mixtures of pentoses and hexoses. Rosa (14) mentions no reasons to suspect that his determinations do not represent the free pentoses present in the tissues and those produced by the hydrolysis of pentosans. However, the numerous determinations of free pentoses and of those hydrolyzed from pentosans, made under a variety of conditions as the present investigations progressed, indicated that the yeast-fermentation method of determining pentoses, as commonly used, is unreliable not only because of the contamination of the fermenting solution with foreign organisms but because of the possibility of the destruction of pentoses or pentose-like reducing material by yeasts. A subsequent and more thorough perusal of the literature indicated that other workers have experienced the same or similar difficulties. For example, Davis and Sawyer (10) mention three species of yeast that ferment pentoses. Armstrong (2) states that, while xylose and arabinose can not be fermented by yeast, they may serve as nutrients for yeast and bacteria. Bokorny (6) reports that yeast can utilize xylose and arabinose. Cross, Bevan, and Smith (8) state that under certain circumstances pentoses in contact with yeast disappear, in one case a decrease of over 50% of arabinose being observed. They state that a condition necessary for this result appears to be a low vitality or starved condition of the yeast.

Schöne and Tollens (15) found that, although pentoses are not fermented by yeasts, they are destroyed by the latter when glucose or other fermentable carbohydrates are present. They found that arabinose with pure yeast showed no loss but, even with care to eliminate foreign organisms, the pentoses disappeared from fermented hydrolyzed jute and beer grains. Of the 10.35% pentose in the jute extract determined by the furfural-HCl-distillation method, only 2.86% remained after fermentation, and from that in the grain extract only 11.46% remained out of 28.15%. They suggest the possibility that the true pentoses (xylose and arabinose) are not fermented but that other and closely related furfural-yielding materials are destroyed.

Cross and Tollens (9) investigated the effect of yeast fermentation on arabinose, xylose, and rhamnose under sterile conditions and with pure cultures of brewing yeast. The sugars were added to yeast in Pasteur's yeast water or to yeast in water plus mineral salts, asparagin, and peptone. In the yeast water the pentoses did not disappear, but in the synthetic medium they did. Losses of 7 to 48% occurred in from 17 to 20 days. No alcohol was formed. They conclude that in an artificial medium poor in organic matter yeast uses pentoses without forming alcohol. From their

own results and the results obtained by some of the other workers in this field, these authors suggest that: (a) it is possible that the pentoses undergo some other fermentation than alcoholic, possibly lactic; (b) under special conditions pentoses may undergo alcoholic fermentation; (c) yeasts may use pentoses as a source of nutriment, as suggested by Bokorny; and (d) there may be substances which will yield furfural by the furfural-HCl-distillation method and which may be destroyed by yeast fermentation but are not pentoses.

Conrad (7) has reported that the pentoses can be determined only approximately by the yeast-fermentation method.

During the course of this investigation it was found that the action of bacteria could be largely eliminated by making the solution 8% alcoholic at the time of adding the yeast. But even this change does not make the method reliable, because, judging from the results secured, some of the common varieties of yeast, such as may be present in Fleischmann's yeast cake, may be able to destroy pentose. This difficulty might be overcome by utilizing, in pure culture and under sterile conditions, strains of yeast which have been found to lack the power of destroying pentoses. In utilizing the method, duplicates for each determination should be run to which known quantities of glucose and pentose have been added. If the added glucose is not entirely fermented and the pentose recovered, the determination must be questioned.

The data secured strongly suggest the existence, in a 1% HCl extract of lemon-peel tissue, of considerable quantities of unfermentable reducing substances which are not pentoses. This was indicated by the fact that in some tests there remained a considerable unfermentable residue when added xylose had completely disappeared. The data obtained, however, compel one to believe that some residue, if not pentoses, then unfermentable reducing material of unknown chemical nature, is determined with at least approximate accuracy by the yeast-fermentation method as used in these determinations. This material is probably derived chiefly from the gum in the endoxerotic lemons and from the cell walls in the healthy ones. It is not considered that the results as given are to be depended upon to show the actual amounts of free pentoses or of those derived from the hydrolysis of pentosans.

It seems probable that there may exist in the healthy lemon a complex which may, for convenience, be designated as X. This complex probably enters into the composition of the cell wall and on hydrolysis yields fermentable sugars chiefly. During the process of gum-formation some factor or factors change most of this portion of X (that hydrolyzable into fermentable sugars) into some substance which yields on hydrolysis unfermentable materials, *i.e.*, *apparent* pentosans. From the tabulated data it appears that this process may be reversible and that part of the gum which yields *apparent* pentosans may be partially changed back again to a form which upon hydrolysis yields fermentable sugars. There probably exists a series

of compounds intermediate between *X* and the gum in the endoxerotic lemon which would yield different proportions of hexoses and *apparent* pentoses upon hydrolysis. Such a fluctuation from one substance or group of substances into another is indicated not only by the analytical data but also by the fact that the gum may very largely disappear from the tissues of the endoxerotic lemons if they are permitted to remain attached to the tree until fully mature, and that it may disappear to a certain extent even after they have been picked. The data as presented, however, show certain uniformities, such as the constancy of the proportion of pentosans to the total carbohydrates, as determined by 1% HCl hydrolysis, in the healthy lemons for all three samples.

Although little uniformity can be observed in the composition of the peels of endoxerotic and healthy lemons so far as other carbohydrates are concerned, there is a uniform difference in polysaccharids. The endoxerotic lemons in all cases show a higher pentosan content and a lower hexosan content. With 1% HCl extractions, the amounts of pentosans are about twice as great in the endoxerotic as in the healthy lemons and the amount of hexosans is only from one sixth to two thirds as great. The difference in hexosan content is not as great as the difference in pentosan content. This would suggest that hexosans in the endoxerotic lemons have been transformed into pentosans. This is in agreement with the statement of Abderhalden (1) that the hexosans are the first plant materials to be changed into pentosans. There is no evidence from the data that the hexoses are the source of the pentosans, as was found by Spoehr for the cacti. The facts that the proportion of carbohydrates present as polysaccharids is nearly constant in both healthy and endoxerotic lemons, and that the endoxerotic lemons show a higher proportion of pentosans and a lower proportion of hexosans than the healthy ones, would again suggest that the gum found in endoxerotic lemons is derived by direct transformation of the hexosans.

The analytical data secured do not clearly answer the question as to whether or not the gum is produced in the endoxerotic lemons as a result of high temperature and a water deficit in the tissues. It is not possible to make comparative tests under conditions in which these factors are controllable, because to date it has not been possible to produce the gum in the lemons under artificial conditions. It should be borne in mind, however, that the gum is formed in the lemons only during periods of comparatively high temperature and at times when other experiments (4, 5) have shown that marked water deficits exist in the tissues. The analyses show that there is a marked increase in polysaccharid and in pentosan content in the endoxerotic fruits, and to this extent they agree with Spoehr's (16) findings. On the other hand, Spoehr found the monosaccharids to be reduced by desiccation, which was not true in this case. The results are hardly comparable, however, for Spoehr was apparently working with pentosans derived from hexoses and not from hexosans. It may also be noted that

the differences which he found in pentosan content due to desiccation and to high temperature are comparatively small.

The problem is an intricate one, and its solution requires a much more comprehensive knowledge of protein-carbohydrate relationships in the cell, of the extent to which water content and temperature relations determine what substances are used directly or indirectly in respiration, and a knowledge of other similar phases of cell activity.

#### SUMMARY

1. A surprisingly large quantity of carbohydrate material is present in the albedo of the lemon peel, from 50 to 62% of the dry matter being composed of reducing material calculated as carbohydrates soluble in 95% alcohol or hydrolyzable in 1% HCl.
2. The polysaccharids hydrolyzed by 1% HCl are approximately constant in amount, composing from 24.7 to 35% of the dry weight of the tissues. The amounts of mono- and disaccharids show a greater variation, from 13.5 to 34.4%, and are not constantly higher in either endoxerotic or healthy tissues.
3. The ratio of the hexosans to the total carbohydrates was found to be always less in the endoxerotic than in the healthy lemons of the same lot, while the ratio of pentosans to total carbohydrates was found to be greater and the ratio of pentosans to hexosans to be much greater in the former than in the latter.
4. The endoxerotic tissues contained from 39 to 63% more pentosans than the healthy tissues.
5. The results indicate that the pentosans were formed at the expense of the hexosans.
6. The yeast-fermentation method as commonly used is not considered to be generally reliable for the determination of pentoses.

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# CHROMOSOME STRUCTURE AND ITS RELATION TO THE CHROMOSOME CYCLE

## II. *PODOPHYLLUM PELTATUM*

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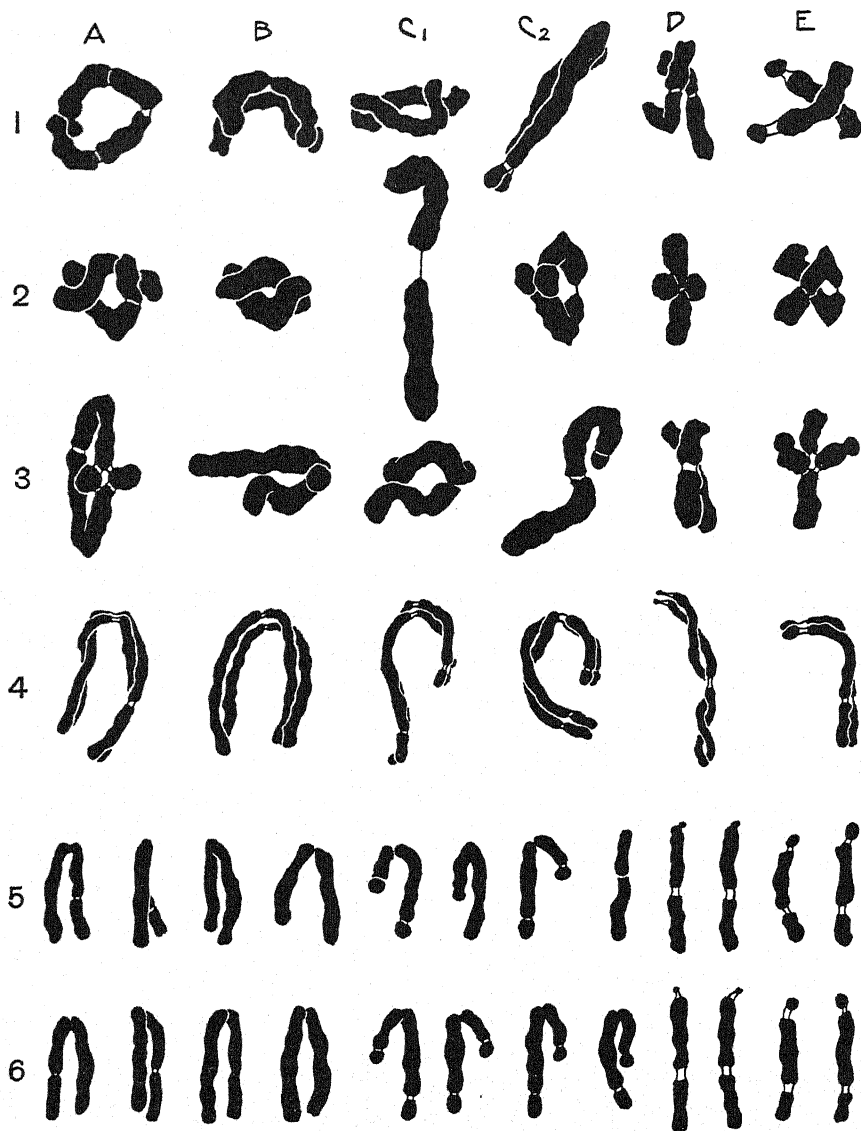
The study here reported was designed to compare the type of chromosome structure in the dicotyledon *Podophyllum peltatum* L. with the chromonemata type described for the monocotyledon *Tradescantia pilosa* (1). In the course of the investigation it was found possible to identify the individual chromosomes through morphological differences. Those findings are incorporated since they help solve the debated question of the chromosome count. This was given by Mottier (4) as 6 to 8 in the pollen mother cells. Later (5) he accepted 8 as the correct number. Overton (6) reported the somatic count as 16. Richards (8) observed 14 chromosomes in about a dozen root tip cells but questioned the correctness of the number. Litardière (2) reported 12 as the diploid chromosome number in material cultivated in the Botanic Garden of Lille. The contrast of his results with those of others caused him to query whether the species concerned did not include several races characterized by different chromosome numbers.

Material for the present paper was collected in Philadelphia and contiguous counties of Pennsylvania during the seasons 1923-1925. Root tips and anthers were sectioned in paraffin. The smear method described by Taylor (11) was also employed in the preservation of the microsporocytes. Observations were made with a Spencer 14JP binocular microscope equipped with 1.40 n.a. achromatic condenser, Zeiss 2 mm. apochromatic objective, and compensating oculars. Drawings were made with the camera lucida at a magnification of 2900 diameters. Text figures 1-14 are reproduced at about 1700 diameters; text figures 15-29, at about 1900 diameters.

### CHROMOSOME FORM AND NUMBER

Microsporocytes of the first maturation division contain 6 tetrads. They are indicated by letters *A-E* in text figures 1-3. Each of the figures represents a total chromosome complex. The bivalents show three types of spindle-fiber attachment, median as in chromosomes *A* and *B*, submedian as in *C*<sub>1</sub> and *C*<sub>2</sub>, and subterminal as in *D* and *E*. Tetrads *D* and *E* are easily distinguished from the others; *A*, *B*, and *C* are more readily confused, when by reason of their compactness, position, or faulty fixation their constrictions are obliterated.

At this stage no outstanding distinctions are apparent between chromosomes *A* and *B*. The median constriction zone may be pronounced as in *A*, text figure 1, or recognized only by its comparative slenderness as in *B* of the same figure. Tetrads *C*<sub>1</sub> and *C*<sub>2</sub> are evidently of one type, although it is possible that differences in the positions of the secondary constrictions



TEXT FIG. 1. Total chromosome complex (*A-E*), microsporocyte, first maturation division. FIGS. 2, 3. Ditto. FIG. 4. Types of metaphase chromosomes, somatic mitosis of root tip. FIG. 5. Members of single anaphase group, somatic mitosis. FIG. 6. Selected chromosomes to represent typical anaphase group.

may exist. In  $C_2$ , text figure 1, a subterminal stricture is noted in the longer arm, while in  $C_2$ , text figure 3, a similar non-chromatic zone appears in the shorter arm. The combination of these on one chromosome is probably the typical condition, as is suggested by a comparison with other tissues. Tetrad  $D$  is best portrayed in text figure 3, although the subterminal fiber attachment is indicated only by a bending of the end of the chromosome. The broad submedian constriction is distinctive of this form. Tetrad  $E$ , text figure 1, exhibits an exaggerated condition of the subterminal zone of fiber attachment. In text figures 2 and 3 the median to submedian secondary constriction is shown.

That the separation of all the dyads is not synchronous is exhibited by text figures 2 and 7. In some cells the longitudinal splitting of the dyads is completed before their separation (selected chromosomes shown in text figs. 9 and 10), although in the majority of cases the split is first seen at a subsequent time.

At the equatorial-plate stage of the second maturation division the specific differences of the chromosomes are more easily distinguished. Text figures 11 and 12 represent the equatorial plates of a single cell. The two groups of chromosomes are not separated by a cell plate but lie in a common cytoplasmic mass. Delimiting of the microspores is accomplished later by the process of furrowing. In text figure 11 the chromosomes are lettered as in text figures 1-6.  $D$  appears telomitic, but, when compared with this chromosome in the somatic mitoses, it is evident that the fiber-attachment zone is so closely subterminal as to be obscured.

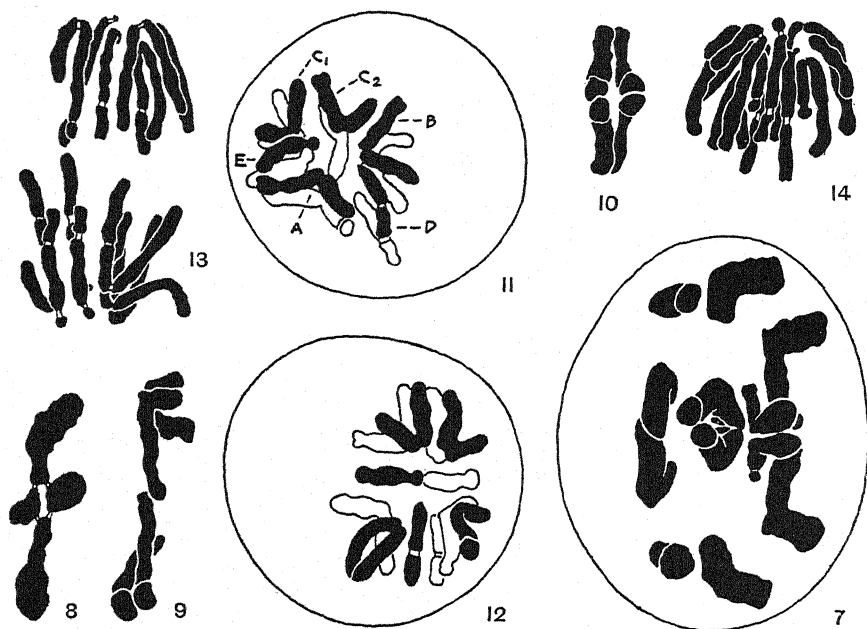
During the somatic mitoses the chromosome forms are most easily recognized. Selected metaphase types are shown in figure 4. In all cases the fiber attachment is as described for the maturation divisions. Chromosome  $D$  clearly exhibits the slightly subterminal non-chromatic zone of fiber attachment. Type  $A$  differs from  $B$  in the possession of a secondary stricture in one of the arms.  $C_1$  and  $C_2$  are apparently similar, with secondary subterminal constrictions in each of the arms. The assumption of similarity is based on the observance of, at the most, three of this type in a single cell. In other cells some of these chromosomes disclose none or only one of the secondary constrictions. However, as was suggested by Taylor (13), failure to demonstrate these structures can not be accepted as positive evidence of their absence. The total complex of a single anaphase group is presented in text figure 5. Text figure 6 is composed of 12 selected chromosomes to illustrate what is perhaps the normal complement. The disposition of the anaphase chromosomes can be seen in either the portion of the cell shown in text figure 13 or the single polar group portrayed in text figure 14.

#### CHROMOSOME STRUCTURE

Bivalents preserved by the smear method are interpreted in text figures 15 and 16. Although the former of these creates effectively the impression



of a body composed of alternating discs or bands of chromatic and achromatic material, the latter figure shows the relation of the striae to the coiled chromatic threads. That the chromonemata should exist at this time is evidenced by text figure 17 of the strepsinema of the first-maturation-division prophase. Critical preservation of this stage is difficult both by the smear and by the paraffin methods. Occasionally, however, free ends of intertwined homologues show themselves to be longitudinally divided, the split separating parallel spiral threads. This is what would be predicted from a consideration of the longitudinally split anaphase and telophase chromosomes of the somatic mitoses.



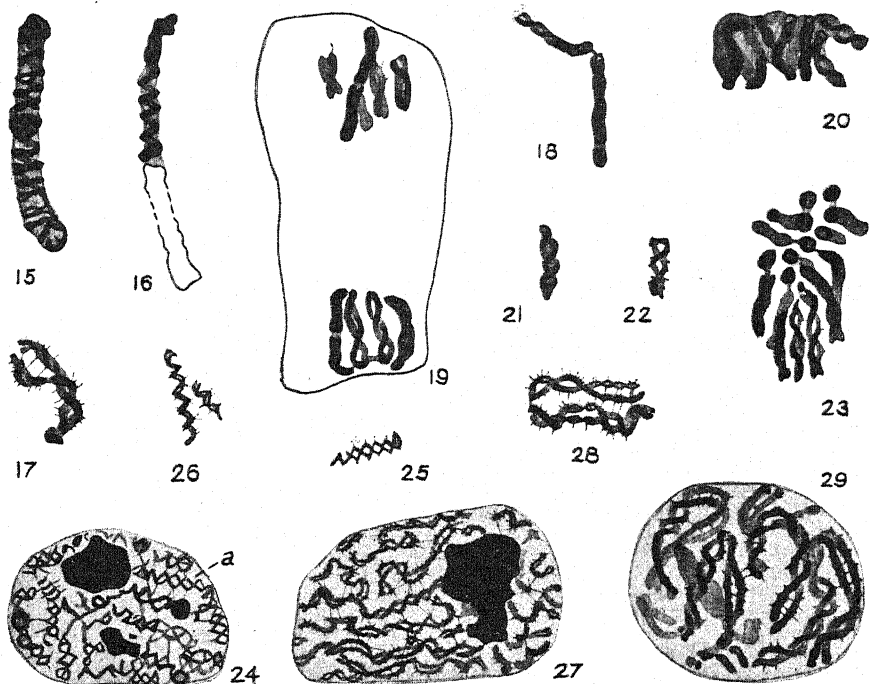
TEXT FIG. 7. Anaphase, first maturation division, microsporocyte. FIG. 8. Tetrad with pronounced constrictions. FIGS. 9, 10. Dyads longitudinally split prior to their separation. FIGS. 11, 12. Equatorial plates, single microsporocyte of second maturation division. Chromosomes of figure 11 lettered as in figures 1-6. FIG. 13, 14. Anaphases, somatic mitosis.

The structure of these anaphase chromosomes appears essentially like that described for *Tradescantia* (1). The chromatic material exists as a pair of chromonemata imbedded in an achromatic matrix which limits the chromosome and forms the material of the constriction zones. In all the figures of this paper, such as text figures 1-6, these zones are to be so regarded and not as paired threads connecting the chromatic material. Text figure 18 shows an anaphase chromosome, one arm of which lies free from the group and exhibits strikingly the coiled threads. A section of an anaphase

cell is interpreted in text figure 19; anaphase groups are shown in text figures 20 and 23. Where seen in end view the chromosome outline is circular, with the chromatic material peripherally disposed. Text figures 21 and 22 are inserted because of the diagrammatic quality of the fragments shown. No polar clumping of the chromosomes was observed, although lateral approximation occurs and anastomosing is often quite pronounced.

With the construction of the daughter nuclei chromosome detail was largely obscured. In the ensuing prophase the double spirals are occasionally observed as shown at *a*, text figure 24, and in figure 25 from another cell at a similar stage. More often a single coiled thread or a pair of tightly interlaced halves are seen (text fig. 26). I am inclined to regard the apparently single thread in the same way as the similar coil noted in text figure 16. Improper preservation has permitted lateral approximation of the halves to the degree of obliterating evidences of duality.

At a later stage the intertwined coils are pronounced and show an in-



TEXT FIG. 15. Tetrad exhibiting chromatic and achromatic substances. FIG. 16. Same (one dyad in outline), showing chromonema. FIG. 17. Strepsinema, prophase, first maturation division. FIG. 18. Anaphase chromosome, somatic mitosis. This and all subsequent figures are from root-tip cells. FIGS. 19, 20. Lateral views late anaphase. FIGS. 21, 22. Detail from stage similar to that of figure 20. FIG. 23. Semi-polar view, anaphase. FIG. 24. Early prophase. FIGS. 25, 26. Chromonemata of early prophase chromosomes. FIG. 27. Later prophase. FIG. 28. Chromosome of late prophase, showing new chromonemata. FIG. 29. Late prophase.

crease in diameter (text fig. 27). Anastomoses proceed from the surfaces of the threads, traversing the inter- and intra-chromosomal spaces. Such processes are seen in text figure 28, representing a chromosome of the *A* type which shows the constriction in one of the arms. At several places in the halves of this chromosome the presence of the new chromonemata was observed. The spirals, as portrayed, are quite distinct and intertwined, suggesting an earlier origin. However, this was not traced, and, if it is within the limits of microscopic visibility, perhaps will not be until more exact fixation allows a critical analysis of the delicate spireme of the early prophase. Text figure 29 shows the chromosomes just prior to the dissolution of the nuclear membrane, at a time when the spindle zone is much denser and stains more deeply than the remainder of the cytoplasm.

### DISCUSSION

The chromosome count of the present paper (diploid 12, haploid 6) is in harmony with that given by Litardière (2) but varies from the reports of the previous American workers. Taylor (12) suggested that some divergences in reports of chromosome numbers might be attributed to misinterpretation of deeply constricted forms. Such an explanation is evidently applicable to the species in question, and perhaps especially concerns the *A-B* chromosomes, whose arms when widely separated by a broad median split might easily be mistaken for a pair of rod-like structures. The constrictions may be regarded as persistent morphological characteristics since they occur in the chromosomes of all the tissues studied. They are preserved with the greatest difficulty in the compacted homologues of the first maturation division, but enough good examples were encountered to prove that they can be demonstrated under proper conditions. They were not figured by Mottier (4, 5), Strasburger (10), or Overton (6), who made extensive studies on the pollen mother cells.

From such chromosomes as the pair shown in text figure 8 and chromosomes *A* and *E* of figure 3 the suggestion is offered that the constrictions form admirable places for segmental interchange between homologues. That the necessary parasynapsis could occur is evidenced by figure 17. This figure shows that the synaptic mates are longitudinally divided, a condition presumably traceable to the preceding mitosis. Unfortunately, critical early prophase stages were not obtained, but figure 17 would indicate a condition analogous to that reported by Robertson (9) of parasynapsis of longitudinally divided chromosomes of the Tettigidae (Orthoptera).

Since the homologues are dual structures, they pair to form a four-parted body or *tetrad*. The adoption of the term seems warranted despite the fact that at a later stage the split between the halves of the dyad may be completely obliterated as in text figure 16, or only slightly suggested as in parts of text figure 15.

The history of the chromonemata of the dyad in relation to the external

appearance of the longitudinal split (text figs. 9, 10) has not been traced in this plant, chiefly because of failure to secure the ideal combination of fixation and stain. Two methods by which the split might be effected are suggested. In one case the line of fracture would traverse the coils of the chromonemata, segmenting them into a number of disconnected pieces. Such an irregular process is less in harmony with the theory of the linear arrangement of genes than is the other method. The split would there follow the line of demarcation established in the preceding cell generation. An untwisting process would occur within the dyad, which as a whole might be regarded as a sort of intranuclear nucleus. The external symptoms of the splitting, the longitudinal cleft of text figures 9 and 10, would be a subsequent process, restricted to the achromatic material.

Since in its major details the somatic mitosis was observed to be similar to that described for *Tradescantia*, attention is restricted to corroboration of some of the more critical phases of the chromosome cycle. Of prime interest is the question of duality of the anaphase and telophase chromosomes. Proof of this duality in *Podophyllum* rests on the following observations. Sometimes the chromatic material appears in the form of a pair of intertwined spiral threads. When the chromonemata can not be traced, the moniliform outline of the chromosome or the indented ends suggest the concealed doubleness. These observations have been countered by those of Overton (7) and Litardière (2, 3). The former saw irregular vacuolation of the anaphase chromosomes occurring in their passage from the equatorial plate to the poles. Litardière (3) figured an anaphase chromosome (his fig. 268) which he believed gave effectively the impression of containing a chromatic spiral thread because of transverse bridges separating a series of axial vacuoles.

In *Tradescantia* I figured the changes by which anastomosing between the coils of the spiral could transform the dyad with its very definite and precise type of organization into a most irregularly vacuolated body. It was thus shown how vacuolation rather than the spiral aspect could be the illusion. The degree to which the anastomosing proceeds is certainly influenced by the speed of penetration of the fixative. This was illustrated by a comparison of the chromosomes of the first maturation division of *Tradescantia* preserved by the smear and by the paraffin methods. Detail was lost in the sectioned material, the bivalents presenting a homogeneous surface rather than the coiled threads seen in the smears. The loss of detail was evidently due to the spreading of the chromatic material within the limiting membrane of the chromosome. Such activity was possible because of the highly viscous, mobile chromatic threads.

Although poor penetration of the fixative and the attendant chemical irritation prior to death causes the chromatic material to undergo extensive changes, it is not unlikely that a certain amount of anastomosing may represent a normal procedure. Surface projections from the chromonemata

are shown in text figures 23-29 of this paper. Litardière (2) likewise saw the anastomoses on the anaphase chromosomes of *Podophyllum*. He says, page 467:

. . . cette espèce, contrairement aux assertions d'Overton (1909), montre bien des anastomoses entre les diverses bandes télophasiques dans des noyaux présentant tous les caractères d'une excellente fixation, de telle sorte que la production des anastomoses paraît réellement un phénomène général à cette période; . . . Il ne faut pas oublier que les chromosomes sont des éléments vivants, formés d'une substance plus ou moins visqueuse, et il est fort compréhensible qu'il puisse s'établir entre eux des connexions autrement que par un processus purement mécanique.

If then the thread is viscous, if the pseudopodium-like processes are normal outgrowths, it seems justifiable to assume that not only adjacent chromosomes but also halves of the same one will be bridged. In this way the appearance of a vacuolated structure could arise.

#### SUMMARY

1. The chromosome count of *Podophyllum peltatum* L. is  $x = 6$ ;  $2x = 12$ .
2. The chromosomes can be distinguished by specific morphological characteristics. They fall into three groups, those with median, those with submedian, and those with subterminal spindle-fiber attachments. Four members of each group are seen in somatic cells. Minor distinctions of form and of positions of the secondary constrictions are noted in the text.
3. In the prophases of the first maturation division evidence is presented of the side-by-side pairing of longitudinally divided homologues.
4. Somatic chromosomes of the anaphases and telophases show duality of chromatic elements which exist as intertwined threads or chromonemata. They are imbedded in an achromatic matrix which forms the material of the constriction zones.
5. The chromonemata perhaps originate in the halves of the prophase chromosomes and are not designed to separate until the anaphases of the succeeding cell generation.

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## LATE FROST INJURY TO SOME TREES IN CENTRAL KENTUCKY

ANSEL F. HEMENWAY

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While mounting some cross sections of 3- and 4-year-old twigs of *Pinus rigida* in the spring of 1922, I noted a rather striking injury to the woody tissue formed early in the previous year. Since then I have examined sections of twigs of two other evergreens, ten species of deciduous shade trees, and ten varieties of fruit trees, growing in or near Lexington, Kentucky. The material used was killed in chrom-acetic fixative, softened in hydrofluoric acid, and cut with a sliding microtome. Sections were stained in iron-alum haematoxylin and safranin.

The literature on late frost injury has recently been reviewed,<sup>1</sup> so it will not be discussed here except to mention one additional reference. Von Schadelin, in his studies of late frost injury in Switzerland,<sup>2</sup> classifies some trees as follows: Least resistant, walnut, ash, beech, fir, oak, spruce; medium resistant, native oak, maple, hornbeam, elm; hardy, white pine and Scotch pine.

It has long been observed that any sort of injury which interrupts the growth of a tree may cause irregularity in cell-formation, and increased production of either wood parenchyma or ray parenchyma, or of both. Mechanical injuries, attacks by fungi, defoliation by insects, injuries caused even by lightning, severe drought, or late frost may occasionally produce false annual rings. For example, *Sequoia sempervirens*, which does not normally show resin canals in wood, may form rows of such canals as a result of wounds. With little or no magnification, these might be mistaken for annual rings. Figure 1, Plate XXVIII, shows typical wound resin canals in *Sequoia sempervirens*.

In the spring of 1921, the temperature in central Kentucky, after a period unusually mild, when many trees were growing and in full leaf, suddenly fell on March 26 to 19° F., and to 20° on April 2. This frost was evidently responsible for the injuries which I observed.

*Pinus rigida* and *Pinus virginiana* were affected similarly. The injury, which occurred after one to three rows of cells had just been formed, is shown by the bending and widening of the rays at that point. This widening is due either to the disjunction of the ray-cell walls or to the proliferation of uniseriate rays until they become tri- or tetraseriate. Sometimes adjacent rays fuse at the point of injury, and occasionally they cease development

<sup>1</sup> Rhoads, A. S. The formation and pathological anatomy of frost rings in conifers injured by late frosts. U. S. Dept. Agr. Bull. 1131. 1923.

<sup>2</sup> Schadelin, W. von. "Beiträge zum Kapitel Spätfrost." Schweiz. Zeitschr. Forstw. 71: 329-334. 1920.

entirely. Between rays, injury is usually evidenced by a dark-staining, rather irregular tangential layer two to four times thicker than the usual tracheid wall, due perhaps to the collapse of a row of injured cells. In other places the injured region is indicated between rays by interruption of the rows of tracheids usually radially arranged, or by the abnormal thickening of the cell walls of one or two rows of tracheids. The injured region seems to have been stretched tangentially and compressed radially. This frost injury was clearly shown in 8-year-old pine twigs, but not so strikingly as in the 3- or 4-year-old twigs.

In the case of *Tilia americana*, injury occurred after one or two vessels or 3 to 5 tracheid cells had been formed radially. Here, adjacent rays may fuse, or rays may cease for a space 6 to 10 cells wide. Between rays, injury is usually evidenced, as in *Pinus*, by a dark-staining tangential layer probably likewise due to the collapse of a row of cells. Then 6 to 12 rows of cells formed after the injury are very irregular, short tracheids, so short, indeed, that their transverse walls are much more in evidence than in the normal wood. Parenchymatous cells also are more common.

The Transcendent crab had grown more than the three aforementioned species, and perhaps therefore shows the injury more strikingly. The frost caused a nearly continuous layer of collapsed cells, followed by many very heavy-walled cells including both vessels and tracheids. These heavy-walled cells are more or less filled with a densely staining material. Rays usually widen at or just outside the line of injury, then narrow to approximately normal width; but about 8 to 10 cells from the point of injury they often re-widen, and occasionally fuse. This abnormal tissue in some places extends for a space of from 18 to 24 cells from the start of injury.

The Early Richmond cherry showed but slight effect from this frost. There were occasional brown spots, perhaps of humin, formed, but apparently no further injury.

The Black Tartarian cherry showed much more serious effect. The injury was farther from the 1920 wood than in the Early Richmond. One or two rows of vessels and 6 to 8 rows of tracheids had been formed before the frost occurred, and the fact that the tree had made more growth was perhaps responsible for its being subject to severer injury. Fusing of adjacent rays just outside the line of injury is common, or rays one cell wide may become 2 to 5 cells wide, or rows of parenchymatous cells one or two cells in width may extend tangentially from one ray to another 4 to 8 cells away. At the line of injury, rays may cease or they may split up into two or three rays. A few vessels and many parenchymatous cells are filled with a densely staining material, perhaps humin.

Maiden Blush apple shows injury like that of Transcendent crab, but less marked and usually confined to one side of the twig. Yellow Transparent apple, Beurre Clairgeau pear, Elberta peach, *Quercus nigra*, and *Fraxinus americana* all showed some injury; in fact, several-year-old twigs on Elberta peach were dead, perhaps from this frost.



Early Crawford peach, Kieffer pear, *Juglans nigra*, *Carya illinoensis*, *Aesculus glabra*, *Cercis canadensis*, *Acer negundo*, *Acer rubrum*, *Celtis occidentalis*, *Picea rubra*, and *Broussonetia papyrifera* showed little if any injury to the xylem cells in 2- to 4-year-old twigs, though most of the year-old twigs of *Celtis occidentalis* were dead, perhaps from this frost.

#### SUMMARY

1. Following a late frost in the spring of 1921 at and near Lexington, Kentucky, 2- to 4-year-old twigs of three evergreens, ten species of deciduous shade trees, and ten varieties of fruit trees were examined for evidences of injury.

2. Injury was evidenced by the presence of false annual rings due to the collapse of from one to three rows of cells, a widening of rays and fusing of adjacent rays, an increase of parenchymatous tissue, the production of short, thick-walled tracheids, and deposits of densely staining material, perhaps humin.

3. The following table shows comparative injuries as evidenced by the condition of 2- to 4-year-old twigs:

<i>Little or No Injury</i>	<i>Injury Slight but Apparent</i>	<i>Injury More Severe</i>
Early Crawford peach	Beurre Clairgeau pear	Transcendent crab
Early Richmond cherry	Maiden Blush apple	Black Tartarian cherry
Kieffer pear	Yellow Transparent apple	<i>Tilia americana</i>
<i>Juglans nigra</i>	Elberta peach	<i>Pinus rigida</i>
<i>Carya illinoensis</i>	<i>Fraxinus americana</i>	<i>Pinus virginiana</i>
<i>Aesculus glabra</i>	<i>Quercus nigra</i>	
<i>Cercis canadensis</i>		
<i>Acer negundo</i>		
<i>Acer rubrum</i>		
<i>Celtis occidentalis</i>		
<i>Broussonetia papyrifera</i>		
<i>Picea rubra</i>		

Inasmuch as through frost injury trees are made susceptible to attacks of bacterial and fungous diseases, further studies of this nature, in regions where late frosts are common, would be profitable.

#### EXPLANATION OF PLATE XXVIII

FIG. 1. *Sequoia sempervirens*, low power, showing false annual ring with traumatic resin canals.

FIG. 2. *Pinus rigida*, high power, showing bending and widening of rays, and dark line probably indicating a tangential row of collapsed cells.

FIG. 3. *Tilia americana*, high power, showing tangential line of collapsed cells, and irregular cell arrangement beyond.

FIG. 4. Transcendent crab, low power, showing comparatively more growth before the injury and more extensive injured area. It also shows fusing of the rays.

FIG. 5. Transcendent crab, high power, showing widening and fusing of the rays, and rewidening and more extensive injury beyond.

FIG. 6. Black Tartarian cherry, high power, showing fusing and widening of rays.

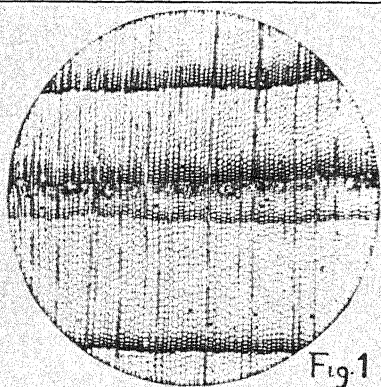


Fig. 1

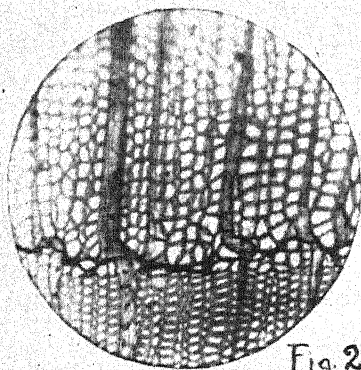


Fig. 2

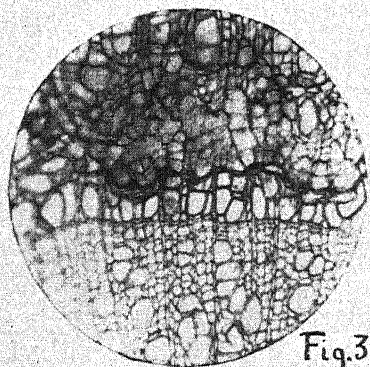


Fig. 3

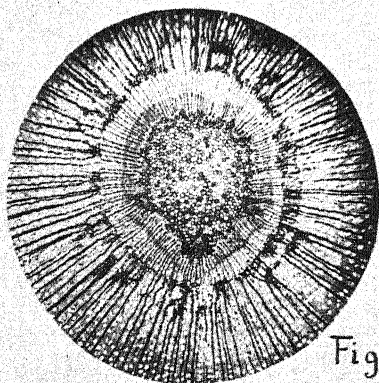


Fig. 4

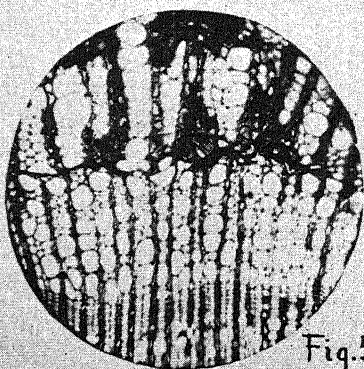


Fig. 5

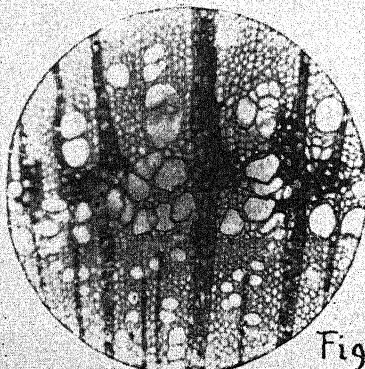


Fig. 6



# CHROMOSOME NUMBERS IN CROP PLANTS<sup>1</sup>

HANNAH C. AASE AND LEROY POWERS

(Received for publication March 22, 1926<sup>2</sup>)

The rapid progress in genetics has awakened the realization that to understand more clearly the mechanism of heredity a cytological knowledge of the material is essential. Investigations, therefore, to determine the chromosome number of parents of cereal hybrids have been given considerable attention during the past three years. This paper is a report of counts made in 1924 and 1925.

The material, excepting that of *Hordeum murinum*, *H. jubatum*, and *Arrhenatherum elatius*, which was obtained from the college campus, was collected from the cereal nursery or the botanical greenhouse. Carleton's and Clark's classifications as far as possible were used in listing the cereals in table 1. Representative drawings were made of each species, with the exception of the species in the genera *Aegilops* and *Avena*, *Aegilops ovata* serving as representative of the former and *Avena Wiestii* as the 14-chromosome representative of the latter.

All drawings and counts were made from the meiotic stages of the microspore mother cells. Both Belling's iron-aceto-carmin method and the standard paraffin iron-alum haematoxylin method were used in preparation of the anthers. Both gave clear meiotic figures. To eliminate possible error that might occur due to abnormal pollen-formation, check counts were taken from different flowers.

Table 1 is a list of the genera and species in which counts were made. This table shows the haploid chromosome numbers to be: *Triticum* 7, 14, and 21; *Avena* 7 and 21; *Hordeum* 7 and 14; *Secale* 7; *Aegilops* 14; and *Arrhenatherum* 14. Kihara (1919) reports having found 14 chromosomes in *Avena barbata*. Therefore, this genus would have 7-, 14-, and 21-chromosome groups. Previous to this time no report has been found of a species of *Hordeum* having more than 7 chromosomes.

It is interesting to note that in the genus *Triticum* the species of the highest chromosome number have the greatest economic value. Thus, the 21-chromosome wheats, *T. vulgare* and *T. compactum*, are more important commercially than *durum*, *dicoccum*, *turgidum*, and *dicoccoides*, the 14-chromosome species of *Triticum*. The 14-chromosome group also ranks in impor-

<sup>1</sup> Published with the approval of the Director of the Washington Agricultural Experiment Station as scientific paper no. 127, College of Agriculture and Experiment Station; also contribution no. 5 from the Department of Botany, State College of Washington.

<sup>2</sup> Published, at the expense of the authors, out of the order determined by the date of receipt of the manuscript.

tance above *T. monococcum*, which has only 7 chromosomes as the haploid number. Likewise, in the genus *Avena* the varieties of greatest commercial value are in the 21-chromosome group. In *Hordeum*, the counts made so far indicate that the reverse is true, as the cultivated species of barley have only 7 chromosomes, while the wild forms, *H. murinum* and *H. jubatum*, have 14.

TABLE 1. *Summary of Chromosome Numbers in Cereals and Related Forms*

Scientific Name	Variety	Common Name	Haploid No.
<i>Triticum monococcum</i> L.....	Einkorn		7
<i>T. dicoccoides</i> Körn.....		Wild wheat	14
<i>T. turgidum</i> L.....	Alaska	Poulard	14
<i>T. dicoccum</i> Schr.....	Black Winter Emmer	Emmer	14
<i>T. durum</i> Desf.....	Kubanka	Durum	14
<i>T. spelta</i> L.....	Alstrom	Spelt	21
<i>T. spelta</i> L.....	White Spring Spelt	Spelt	21
<i>T. spelta</i> L.....	Bearded Spelt	Spelt	21
<i>T. compactum</i> Host.....	Hybrid 12 <sup>8</sup>	Club wheat	21
<i>T. vulgare</i> Vill.....	Ridit	Common wheat	21
<i>T. vulgare</i> Vill.....	Bluestem	Common wheat	21
<i>T. vulgare</i> Vill.....	Hussar	Common wheat	21
<i>T. vulgare</i> Vill.....	Martin	Common wheat	21
<i>T. vulgare</i> Vill.....	Triplet	Common wheat	21
<i>Avena Wiestii</i> Steudel.....		Abyssinian oats	7
<i>A. brevis</i> Roth.....		Short oats	7
<i>A. strigosa</i> Schreber.....		Rough or sand oats	7
<i>A. sativa</i> L.....	Markton	Common or spreading oats	21
<i>Hordeum spontaneum</i> K. Koch..		Two-row barley	7
<i>H. vulgare</i> L.....	Winter Club	Common six-row barley	7
<i>H. murinum</i> L.....		Wall barley	14
<i>H. jubatum</i> L.....		Squirrel tail	14
<i>Secale cereale</i> L.....	Rosen	Rye	7
<i>Aegilops cylindrica</i> Host.....			14
<i>A. triuncialis</i> .....			14
<i>A. squarrosa</i> .....			14
<i>A. ovata</i> .....			14
<i>Arrhenatherum elatius</i> L.....		Tall oat grass	14

The size of the pollen mother cells of wheat is, generally speaking, found to be directly proportional to the chromosome number. This difference in size shows especially clearly in comparing the 7-chromosome group (fig. 1, Pl. XXIX) with the 14- and 21-chromosome groups (figs. 2, 4, 5, 6, 7, 9, 10). This distinction is not so marked between the 14- and 21-chromosome wheats. These findings conform with those of Sax (1922) that the size of the pollen grain is closely correlated with the chromosome number in the various species of wheat.

Table 2 is a summary of chromosome counts in crop plants and related forms (omitting species counted by the writers), and is complete only in so far as the literature and time available would permit. The outstanding feature in this table is the frequency with which the chromosome numbers of the species within each genus range either in multiples of a certain number or in multiples of a basic number. In *Rubus*, for instance, Longley (1924a)

TABLE 2. · Summary of Chromosome Numbers in Crop Plants and Related Forms

Scientific Name	Common Name	Haploid No.	Authority
<i>Zea Mays</i> . . . . .	Corn	10	Longley, 1924 <i>b</i>
<i>Coix lachryma jobi</i> L. . . . .		10	Longley, 1924 <i>b</i>
<i>Euchlaena mexicana</i> . . . . .	Teosinte	10	Longley, 1924 <i>b</i>
<i>E. perennis</i> . . . . .	Teosinte	20	Longley, 1924 <i>b</i>
<i>Tripsacum dactyloides</i> . . . . .		35	Longley, 1924 <i>b</i>
<i>T. laxum</i> . . . . .		35	Longley, 1924 <i>b</i>
<i>T. pilosum</i> . . . . .		35	Longley, 1924 <i>b</i>
<i>T. lanceolatum</i> . . . . .		35	Longley, 1924 <i>b</i>
<i>Saccharum officinarum</i> . . . . .	Sugar cane	40	Bremer, 1923
<i>S. spontaneum</i> . . . . .		56	Bremer, 1923
<i>Oryza sativa</i> . . . . .	Rice	12	Nakatomi, 1923
<i>Allium Cepa</i> . . . . .	Common onion	16 *	Schaffner, 1898
<i>Musa sapientum</i> "dole" . . . . .	Banana	8	Tischler, 1910
<i>M. sapientum</i> "Rajah Siam" . . . . .	Banana	16	Tischler, 1910
<i>M. sapientum</i> "Kladi" . . . . .	Banana	24	Tischler, 1910
<i>Morus</i> . . . . .	Mulberry	14	Osawa, 1920
<i>Morus</i> . . . . .	Mulberry	42 *	Osawa, 1920
<i>Brassica nigra</i> Koch . . . . .	Black or brown mustard	16 *	Karpechenko, 1922-23
<i>B. "Schnittkohl"</i> L. . . . .	Black mustard	18 *	Karpechenko, 1922-23
<i>B. oleracea</i> L. . . . .	Cabbage, etc.	18 *	Karpechenko, 1922-23
<i>B. rapa</i> L. . . . .	Turnip	20 *	Karpechenko, 1922-23
<i>B. napus</i> L. . . . .	Rape	36 *	Karpechenko, 1922-23
<i>B. Juncea</i> Czern. . . . .		36 *	Karpechenko, 1922-23
<i>Sinapis arvensis</i> L. . . . .	Field mustard	18 *	Karpechenko, 1922-23
<i>S. dissecta</i> Lag. . . . .		18 *	Karpechenko, 1922-23
<i>S. alba</i> L. . . . .	White mustard	24 *	Karpechenko, 1922-23
<i>Raphanus raphanistrum</i> L. . . . .	Wild radish	18 *	Karpechenko, 1922-23
<i>R. sativus</i> L. . . . .	Garden radish	18 *	Karpechenko, 1922-23
<i>Ribes</i> . . . . .	Currant	8	Tischler, 1906
<i>Rubus</i> † . . . . .	Blackberries	7	Longley, 1924 <i>a</i>
<i>Pisum sativum</i> . . . . .	Pea	7	Cannon, 1903
<i>Phaseolus vulgaris</i> . . . . .	Bean	16 *	Wager, 1904
<i>Melilotus alba</i> . . . . .	White sweet clover	8	Castetter, 1925
<i>Gossypium arboreum</i> . . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. sanguineum</i> Tod. . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. roseum</i> Tod. . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. neglectum</i> Tod. . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. cernuum</i> Tod. . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. rudicum</i> . . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. mollisoni</i> . . . . .	Indian and Chinese cotton	13	Denham, 1924
<i>G. peruvianum</i> . . . . .	Egyptian cotton	26	Denham, 1924
<i>G. barbadense</i> . . . . .	Sea Island cotton	26	Denham, 1924
<i>G. hirsutum</i> L. . . . .	American cotton	26	Denham, 1924
<i>G. mexicanum</i> Tod. . . . .	American cotton	26	Denham, 1924
<i>Nicotiana Langsdorffii</i> . . . . .	Tobacco	9	Goodspeed, 1924
<i>N. alata</i> . . . . .	Tobacco	9	Goodspeed, 1924
<i>N. longiflora</i> . . . . .	Tobacco	9	Goodspeed, 1924
<i>N. sylvestris</i> . . . . .	Tobacco	12	Goodspeed, 1924
<i>N. glauca</i> . . . . .	Tobacco	12	Goodspeed, 1924
<i>N. suaveolens</i> . . . . .	Tobacco	12	Goodspeed, 1924
<i>N. glutinosa</i> . . . . .	Tobacco	12	Goodspeed, 1924
<i>N. paniculata</i> . . . . .	Tobacco	12	Goodspeed, 1924
<i>N. acuminata</i> . . . . .	Tobacco	12	Goodspeed, 1924
<i>N. rustica</i> . . . . .	Tobacco	24	Goodspeed, 1924
<i>N. Bigelovii</i> . . . . .	Tobacco	24	Goodspeed, 1924
<i>N. nudicaulis</i> . . . . .	Tobacco	24	Goodspeed, 1924
<i>N. Tabacum</i> . . . . .	Tobacco	24	Goodspeed, 1924
<i>Solanum lycopersicum</i> . . . . .	Tomato	12	Winkler, 1910

\* Diploid chromosome number.

† In the genus *Rubus*, Longley found diploid, triploid, tetraploid, pentaploid, hexaploid, and octoploid species.

divides the species into two major classes: (1) diploid, with 7 as the gametophytic number; (2) polyploid, including triploid, tetraploid, pentaploid, hexaploid, and octoploid forms with gametophytic numbers in multiples of 7. The only apparent exception is in the genus *Brassica*.

In table 1 the basic number is 7, all species counted having 7, 14, or 21 chromosomes as the haploid number. This raises the question whether one species could have developed from another by hybridization. Percival believes the bread wheats, or 21-chromosome wheats, to be of hybrid origin and to have in their parentage the emmer wheats and two *Aegilops* species, *cylindrica* and *ovata*. He derives these conclusions from the fact that the characters found in *Triticum vulgare*, not explainable by the emmer group, can be accounted for by *Aegilops cylindrica* and *A. ovata*. This hypothesis is also upheld by genetical and cytological investigations. Sax (1924) states that the probability of the *vulgare* type of wheat having emmer, *Aegilops cylindrica*, and *A. ovata* in its parentage is in accord with the taxonomic, genetic, and cytological relationship of the wheat species and related genera. From this, it would seem that the *vulgare* type of wheat might have originated from species having a lower chromosome number. Whether a similar hypothesis is plausible for other species listed in table 1 is uncertain, as the work so far is of too preliminary a nature.

The limited amount of work done shows the need for more cytological and genetical investigation. The fact that the crop plants that have been investigated range, as to chromosome numbers, in multiples of some basic number suggests a field of investigation that may lead to a more definite knowledge of their ancestry. By crossing the cultivated species with their wild relatives and keeping records of their genetical and cytological behavior it might be possible to obtain information which would give geneticists a clearer insight into the mechanism of heredity and which would lead to valuable economic results.

Acknowledgments are due Dr. E. F. Gaines for valuable criticisms on the genetical phases of this paper.

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## DESCRIPTION OF PLATES

Stages in the meiotic divisions, microspore mother cells. All figures drawn with camera lucida. Magnification, 1250 diameters.

### PLATE XXIX

FIG. 1. *Triticum monococcum* (Einkorn). Heterotypic equatorial plate, polar view; 7 paired chromosomes.

FIG. 2. *Triticum dicoccoides* (wild wheat). Heterotypic equatorial plate, polar view; 14 paired chromosomes, one pair with chromosomes attached end to end.

FIG. 3. *Triticum dicoccoides* (wild wheat). Heterotypic anaphase, side view; 14 chromosomes approaching each pole. Microspore mother cell considerably flattened.

FIG. 4. *Triticum dicoccum* var. Black Winter Emmer. Heterotypic equatorial plate, polar view; 14 paired chromosomes.



FIG. 5. *Triticum turgidum* var. Alaska. Heterotypic equatorial plate, polar view; 14 paired chromosomes.

FIG. 6. *Triticum durum* var. Kubanka. Heterotypic equatorial plate, polar view; 14 paired chromosomes. Many chromosomes tipped from the normal position.

FIG. 7. *Triticum spelta* var. Bearded Spelt. Heterotypic equatorial plate, polar view; 21 paired chromosomes.

FIG. 8. *Triticum spelta* var. Alstroum. Homoeotypic equatorial plates, polar view; 21 paired chromosomes in each cell of dyad.

FIG. 9. *Triticum compactum* var. Hybrid 128. Heterotypic equatorial plate, polar view; 21 paired chromosomes.

FIG. 10. *Triticum vulgare* var. Ridit. Heterotypic equatorial plate, polar view; 20 paired and 2 single chromosomes.

#### PLATE XXX

FIG. 11. *Avena Wiestii* (Abyssinian oats). Homoeotypic equatorial plate, polar view; 7 chromosomes.

FIG. 12. *Avena sativa* var. Markton. Heterotypic equatorial plate, polar view; 21 paired chromosomes.

FIG. 13. *Hordeum spontaneum* (two-row barley). Heterotypic equatorial plate, polar view; 7 paired chromosomes.

FIG. 14. *Hordeum vulgare* var. Winter Club. Homoeotypic equatorial plate, polar view; 7 chromosomes.

FIG. 15. *Hordeum murinum* (wall barley). Heterotypic equatorial plate, polar view; 14 paired chromosomes. Pollen mother cell flattened.

FIG. 16. *Hordeum jubatum* (squirrel tail). Heterotypic equatorial plate, polar view; 14 paired chromosomes.

FIG. 17. *Hordeum jubatum* (squirrel tail). Heterotypic equatorial plate, side view; 14 paired chromosomes, 3 pairs showing components attached end to end.

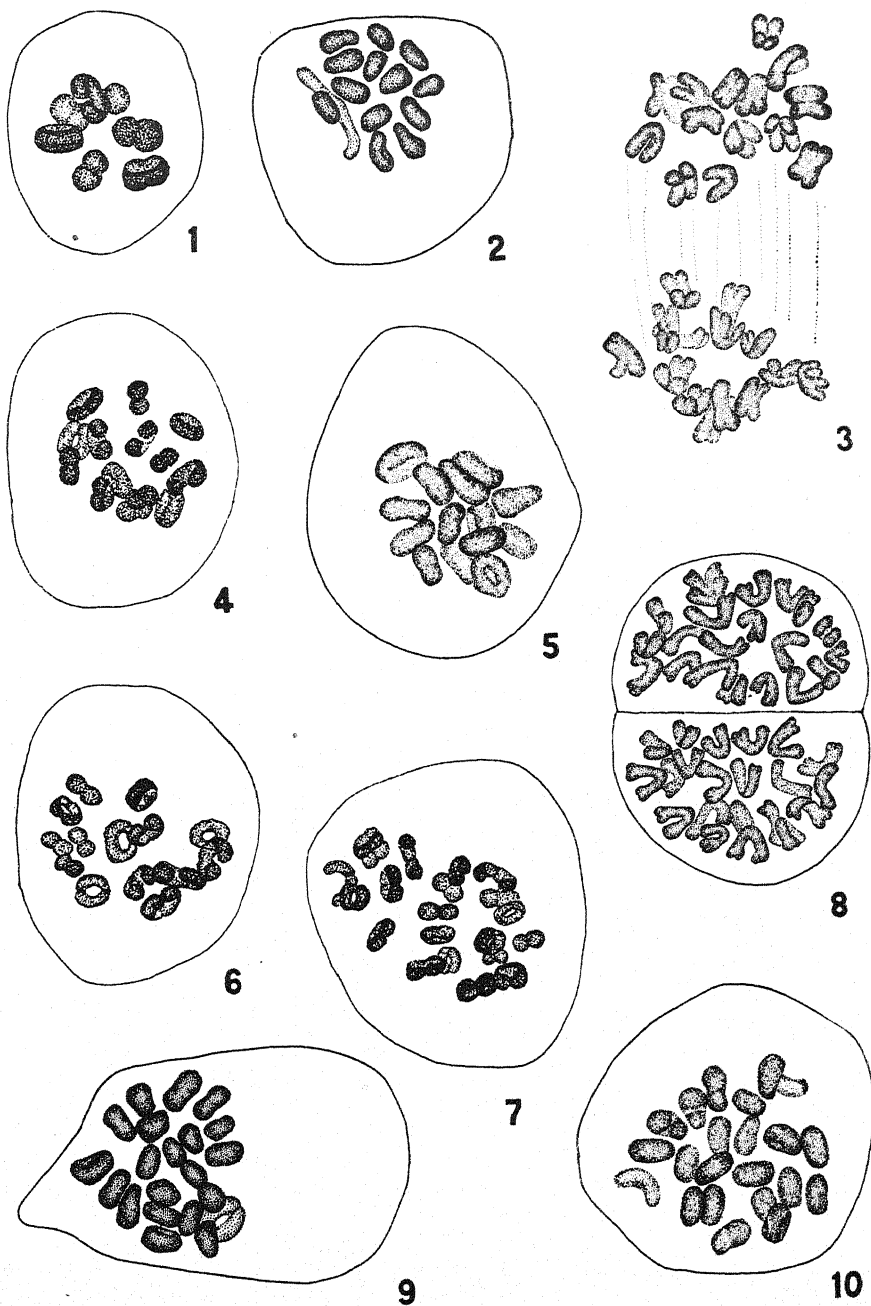
FIG. 18. *Secale cereale* var. Rosen. Heterotypic equatorial plate, polar view; 7 paired chromosomes.

FIG. 19. *Secale cereale* var. Rosen. Homoeotypic equatorial plate, polar view; 7 chromosomes.

FIG. 20. *Aegilops ovata*. Heterotypic equatorial plate, polar view; 14 paired chromosomes.

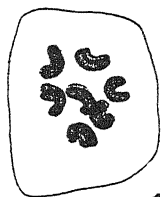
FIG. 21. *Arrhenatherum elatius* (tall oat grass). Heterotypic anaphase, diagonal view; 14 chromosomes approaching each pole.

FIG. 22. *Arrhenatherum elatius* (tall oat grass). Homoeotypic equatorial plate, polar view; 14 chromosomes.

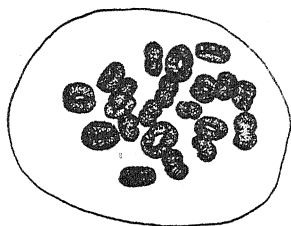


AASE AND POWERS: CHROMOSOME NUMBERS

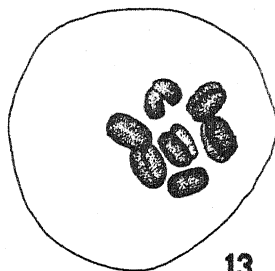




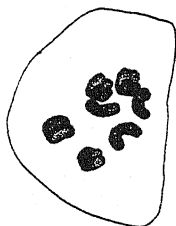
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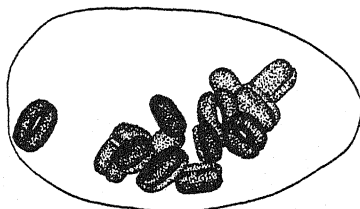
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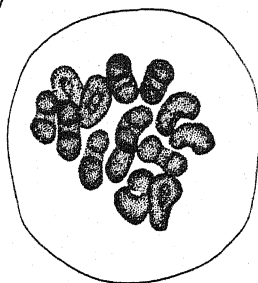
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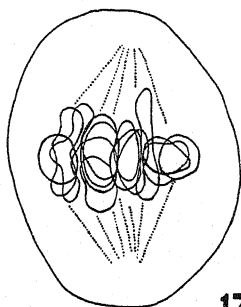
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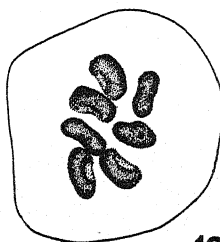
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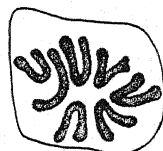
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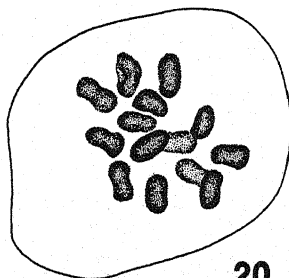
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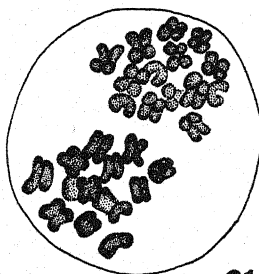
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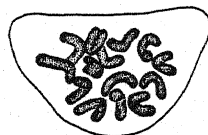
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22



## A HAPLOID WHEAT PLANT<sup>1</sup>

E. F. GAINES AND HANNAH C. AASE

(Received for publication March 22, 1926<sup>2</sup>)

Hybrid 128, *Triticum compactum humboldtii* Kcke., is a prolific and popular winter wheat variety of the Pacific Northwest. It normally is diploid, having 42 chromosomes (21 pairs) in somatic cells. A plant was found in 1925 in the cereal nursery of the Washington Agricultural Experiment Station at Pullman that had but 21 chromosomes. Examination at sporogenesis showed it to be a haploid plant. This plant was vigorous, tillered well, and could not be distinguished from a normal diploid plant until flowering time, when the spreading of the glumes characteristic of sterility drew attention to it (text fig. 1).

The seed which produced this haploid plant was supposed to be a cross between Hybrid 128 wheat and *Aegilops cylindrica*. Seeds from this cross are usually shriveled and weigh from 15 to 25 milligrams, but this kernel was plump and abnormally large, weighing 45 milligrams. Normal seeds of Hybrid 128 weigh from 25 to 35 milligrams, and seeds of *Aegilops* are much smaller.

Evidently the injury to the flower in taking out the anthers or the stimulation of the *Aegilops* pollen caused this seed to develop without the addition of any male chromatin to the nucleus of the egg which formed the embryo. It is possible that both of the male gamete nuclei fused with the maternal endosperm nuclei, which would account for the giant endosperm. At any rate, the seed germinated and produced a plant resembling Hybrid 128 in every respect. It was marked "selfed" in the records as it came into head.

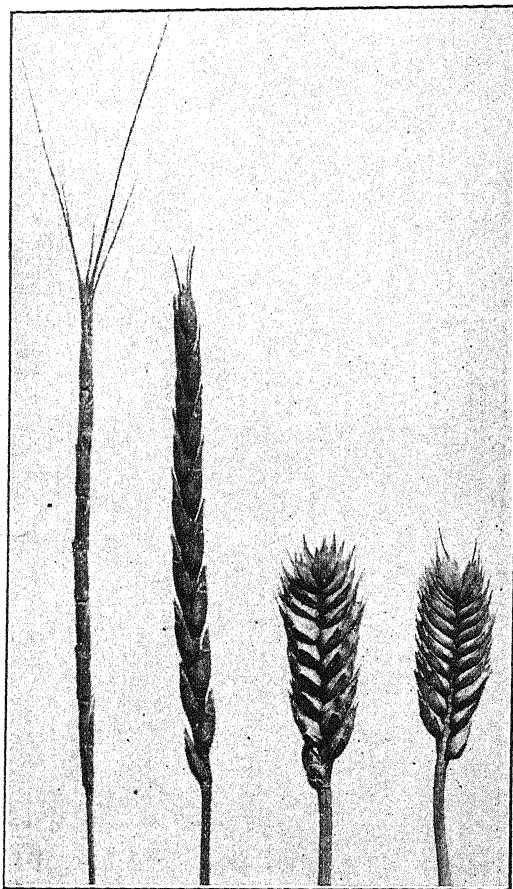
It is not uncommon in hybridizing wheat to break an anther in the process of emasculating the flower, or to introduce pollen by accident from the pistillate parent, in which case a selfed seed may result. About 10 percent of the "hybrid" seeds obtained in wheat prove to be selfed and produce offspring exactly like the maternal parent in subsequent generations.

It was not until the unusual flowering behavior of this haploid plant attracted attention that the notation in the record book was questioned and a cytological study was undertaken. The flowers stood open for 24 to 48 hours, whereas in normal fertilization the time is measured in minutes,

<sup>1</sup> Published with the approval of the Director of the Washington Agricultural Experiment Station as scientific paper no. 129, College of Agriculture and Experiment Station; also contribution no. 6 from the Department of Botany of the State College of Washington.

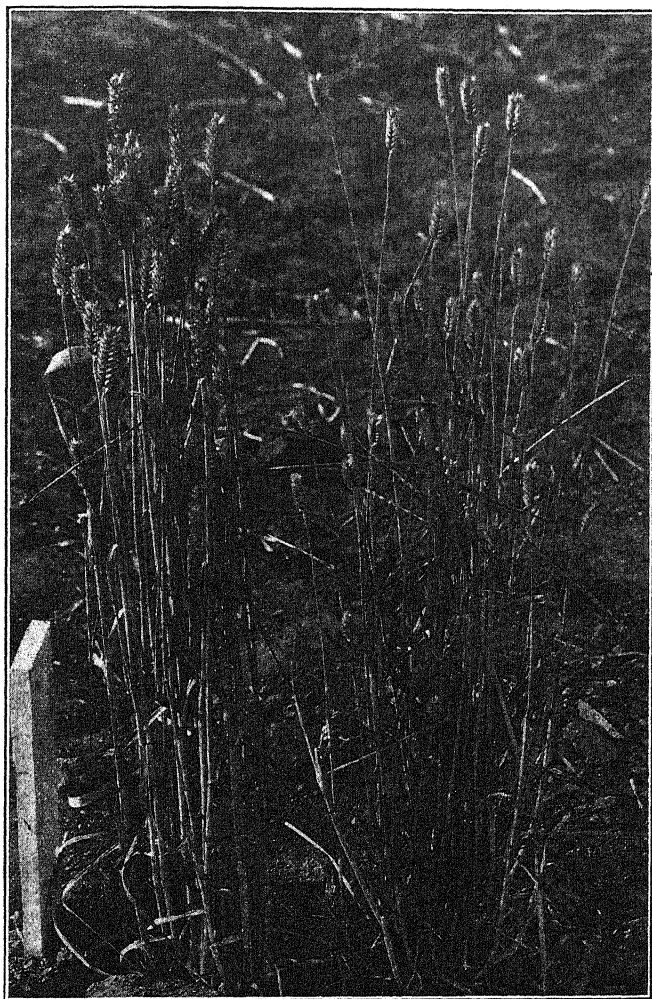
<sup>2</sup> Published, at the expense of the State College of Washington, out of the order determined by the date of receipt of the manuscript.

varying from 5 to 30 depending upon temperature, humidity, etc. As the earlier culms turned yellow, new tillers sprang up from the base of the plant which offered material suitable for chromosome counts. The 49 well-developed heads were examined to see whether sterility was complete. Nine seeds were found, three of which did not germinate. Each head bore from 60 to 80 flowers. Thus a viable seed was produced about once for each 500 flowers. Stated in percentage, the plant was 99.8 percent sterile.



TEXT FIG. 1. Left, a normal plant of Hybrid 128 having 42 chromosomes (21 pairs) in somatic cells. The heads are completely fertile. Right, the haploid plant of Hybrid 128 having but 21 single chromosomes in somatic cells. It is 99.8 percent sterile, but in vegetative vigor and plant characteristics exactly like a normal plant.

Sterility as nearly complete as this is common in wide crosses, but it could not have been an  $F_1$  (text fig. 2). The  $F_1$  head is twice as long as the haploid Hybrid 128, and the glumes are very different. The cytological



TEXT FIG. 2. Left to right: *Aegilops cylindrica*, Hybrid 128  $\times$  *A. cylindrica* F<sub>1</sub>, Hybrid 128, haploid sporophyte of Hybrid 128 that arose from a seed supposed to have resulted from pollination by *Aegilops cylindrica* pollen. It is sterile and has but 21 single chromosomes in somatic cells.

record showing 21 single chromosomes corresponds with the gametic number contributed by the mother plant, which leaves no doubt that this plant is a haploid sporophyte similar to the Jimson weed plants described by Blakeslee, Belling, Farnum, and Bergner,<sup>3</sup> and the tobacco plants reported by Clausen and Mann.<sup>4</sup>

<sup>3</sup> Blakeslee, A. F., Belling, J., Farnham, M. E., and Bergner, A. Dorothy. A haploid mutant in Jimson weed, "*Datura stramonium*." *Science* n. ser. 55: 646, 647. 1922.

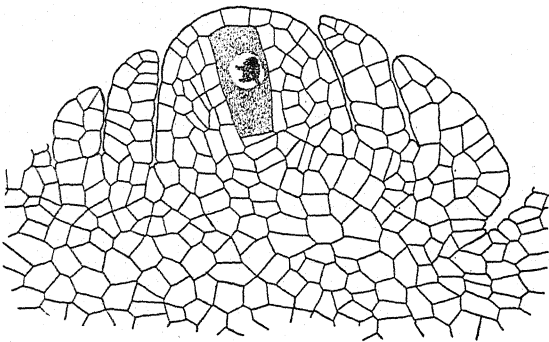
<sup>4</sup> Clausen, Roy E., and Mann, Margaret C. Inheritance in *Nicotiana tabacum* V. The occurrence of haploid plants in interspecific progenies. *Proc. Nat. Acad. Sci.* 10: 121-124. 1924.



## CYTOLOGICAL OBSERVATIONS

The stamens and pistils were fixed either in iron-aceto-carmine on the slide, or in Allen's modification of Bouin's solution, sectioned, and stained in Heidenhain's iron-alum haematoxylin. Each method supplements the other quite advantageously. The former method causes considerable swelling of the material while the latter is more conducive to shrinkage. Text figs. 4 *C* and 5 *D* illustrate the swelling effect of the iron-aceto-carmine. The size of the chromosomes varies somewhat in both preparations, depending upon the progress of mitosis, especially if there is a delay in the sequence of phases. All drawings were made with the aid of the camera lucida.

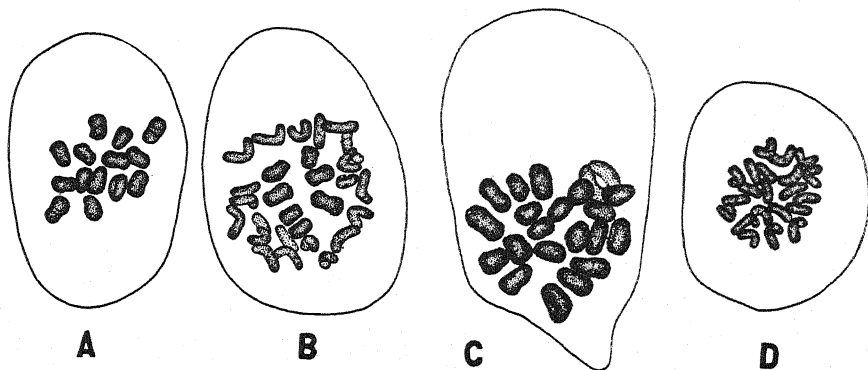
The young stamens and pistils are normal in appearance, but there is evidently a retardation in tetrad-development, as larger anthers and ovaries show divisions of mother-cell nuclei than is the case in a diploid plant, and during a longer period of time. A small number of anthers occurs in which vegetative tissue replaces sporogenous tissue in one to all of the locules. The general morphology of the young ovules examined indicates no abnormality (text fig. 3).



TEXT FIG. 3. Ovule, from transverse section of ovary.  $\times 315$ .

The pollen mother cells average smaller than in diploid plants. The early prophase are marked by the usual events. Synizesis occurs and also the "second contraction." No sufficiently intensive study has as yet been made of the interesting problems that, no doubt, are presented by the prophase to warrant discussion at this time. In the late prophase and in the metaphases of the first division there are 21 distinct unpaired chromosomes in each spore mother cell of both anthers and ovules (text figs. 4 *D* and 5 *A*, *B*) as compared with the 21 pairs in the diploid sporophyte of Hybrid 128 (text fig. 4 *C*), or the 14 pairs in *Aegilops cylindrica* (text fig. 4 *A*). Occasionally two and rarely more chromosomes pair or at least adhere end to end (text fig. 5 *A*). Consistent pairing, such as is commonly observed in  $F_1$  hybrids of 21-chromosome (haploid number) wheats and *Aegilops cylindrica* (14 chromosomes) does not occur (text fig. 4 *B*).

The chromosomes sometimes appear in the typical equatorial-plate formation (text fig. 4 *D*), but they are more frequently found distributed over the entire spindle (text fig. 5 *A*, *B*). This latter behavior makes it

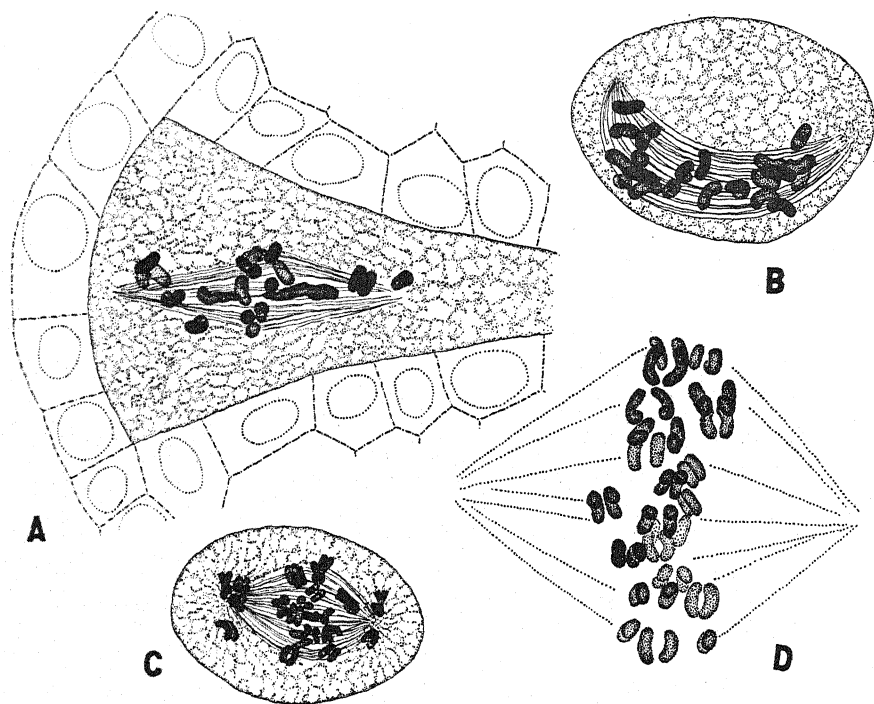


TEXT FIG. 4. Heterotypic equatorial plates in pollen mother cells, polar view.  $\times 1250$ . *A*, *Aegilops cylindrica*, 14 paired chromosomes. *B*, Red Hussar wheat  $\times$  *Aegilops cylindrica*, 7 paired and 21 unpaired chromosomes. *C*, Hybrid 128 wheat, diploid, 21 paired chromosomes. (Fixed in iron-aceto-carmin, hence larger size.) *D*, Hybrid 128, haploid, 21 unpaired chromosomes.

difficult to distinguish the metaphases, if they can be so called, from the early anaphases. The spindle extends lengthwise through the cell (text fig. 5 *A*) or forms a crescent along one side (text fig. 5 *B*).

During the later anaphases the procedure differs radically in the individual spore mother cells. The univalent chromosomes may go to the opposite poles in approximately equal numbers, or a larger number may go to one pole than to the other (text fig. 6 *B*). Chromosomes frequently wander in the cytoplasm and fail to be included in the daughter nuclei, but form small independent nuclei (text fig. 6 *A*). Sometimes chromosomes seem to become entangled, the result being stretched chromosomes extending between the nuclei; and this feature may become intensified until the chromosome groups are apparently torn apart into more or less irregular masses, often with connecting strands; or the entire chromosome group may cohere in an irregular mass in the center of the cell.

In a smaller number of spore mother cells each univalent chromosome splits lengthwise and the halves proceed in an orderly manner to opposite poles, simulating a homoeotypic division (text fig. 5 *D*). Or the halves of only some of the split chromosomes go to opposite poles while the two halves of each of the other chromosomes cohere and go together to one or the other pole (text fig. 5 *C*), so that the nucleus of each cell of the resulting dyad contains a mixture of entire univalent chromosomes and halves of univalent chromosomes. The state of separation of the two halves of each chromosome in the anaphases may depend largely upon the position

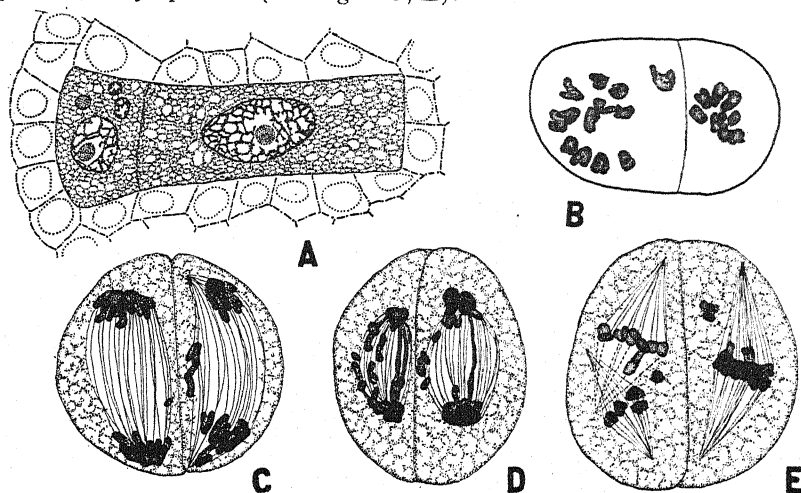


TEXT FIG. 5. Heterotypic metaphase-anaphases, side view.  $\times 1250$ . *A*, megaspore mother cell. Unpaired chromosomes going to poles. Suggestion of end-to-end pairing. *B*, pollen mother cell. Unpaired chromosomes going to poles. Curved spindle typical of a large number of pollen mother cells. *C*, pollen mother cell. The unpaired or univalent chromosomes lying near the center of the cell have split. *D*, the halves of all the split univalent chromosomes are separating in the first division and will go to opposite poles. (The large size of the chromosomes is due to the iron-aceto-carmin fixation.)

of the chromosomes at the time the split occurs. When the lengthwise splitting is initiated it progresses almost simultaneously in all the chromosomes of the cell, and it is probable that, if a dividing chromosome lies near the center of the spindle, its halves are more likely to separate than if it lies nearer to the pole.

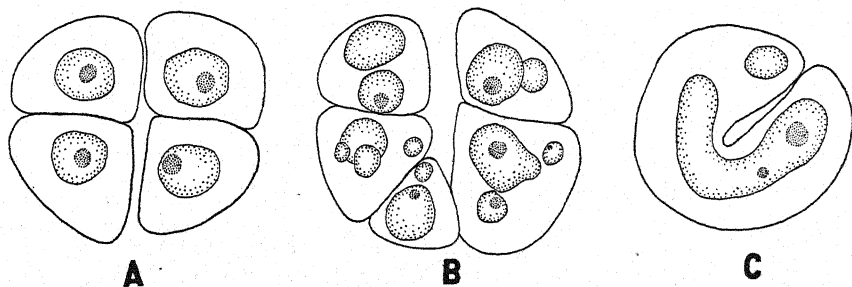
The procedure in the second division (corresponding to the homoeotypic division) depends largely upon what has taken place in the first. If the chromosomes were distributed nearly equally or even quite unequally to the two poles in the first division, the second division may progress with seemingly little disturbance, each chromosome dividing as in a regular homoeotypic division. The resulting tetrad may appear quite normal (text fig. 7 *A*), but usually the four spores are unequal in size and the cytoplasm may be dotted with additional nuclei formed by chromosomes which had strayed in the first or second division. Occasionally more than four spores are formed (text fig. 7 *B*) in consequence of the straying of chromosomes or groups of

chromosomes. This state of polyspory is often further augmented by supernumerary spindles (text fig. 6 *C, E*).



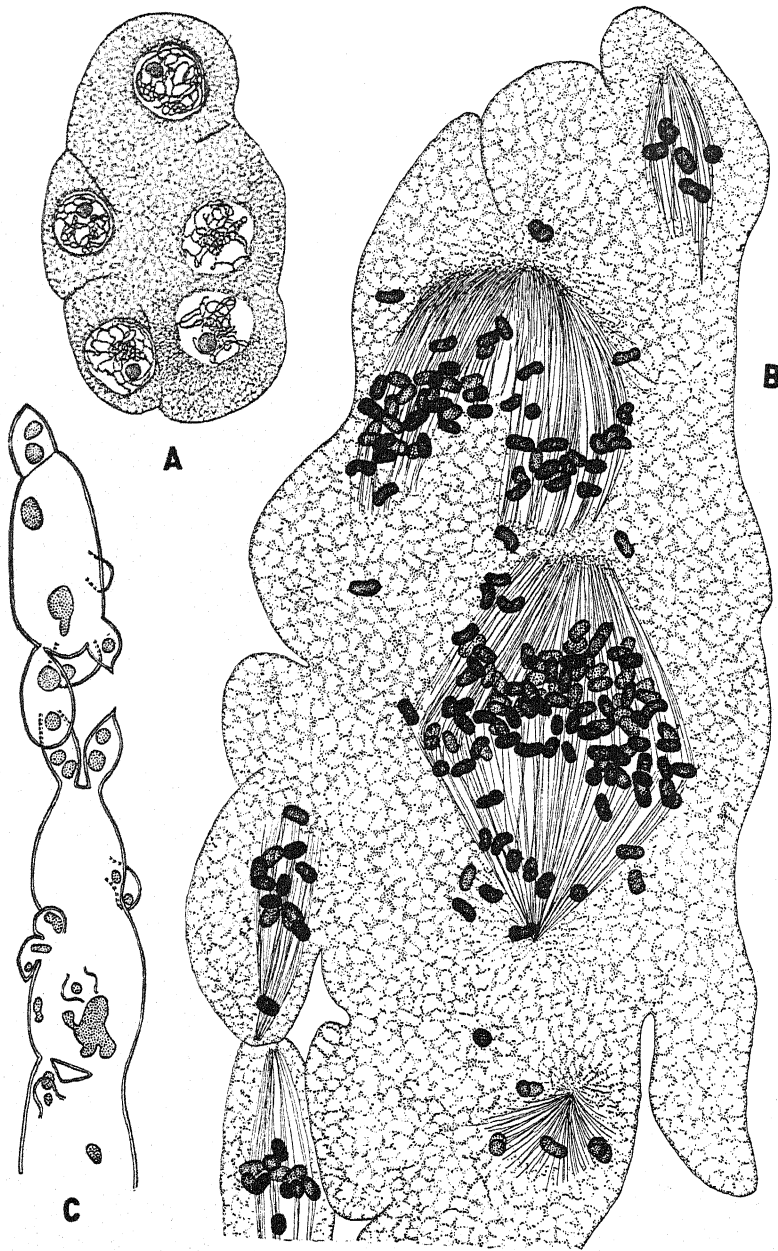
TEXT FIG. 6. *A*, dyad from ovule. Additional small nuclei in outer cell due to straying chromosomes.  $\times 650$ . *B*, dyad from anther, homoeotypic equatorial plates. Chromosomes unevenly distributed and irregularly oriented. *C*, homoeotypic anaphase. Two parallel spindles in cell at left. *D*, distortion and lagging of chromosomes. *E*, additional spindle. Two chromosomes in cell at right failed to become oriented on the spindle.  $\times 1250$ .

If the chromosomes were left in conglomerate masses by the first division, the second division may be extremely chaotic (text fig. 6 *D*). Tetrads result, but the spores usually contain more irregularly shaped nuclei. Rarely irregular masses of chromatin remain in the center of the cell, and tetrad-formation fails (text fig. 7 *C*).



TEXT FIG. 7. "Tetrads."  $\times 1250$ . *A*, normal in appearance. *B*, five spores, and each spore containing accessory nuclei. *C*, failure in tetrad-formation. The large nucleus is the result of the coherence of most of the chromosomes in the center of the cell. Cytoplasm partly divided by furrowing.

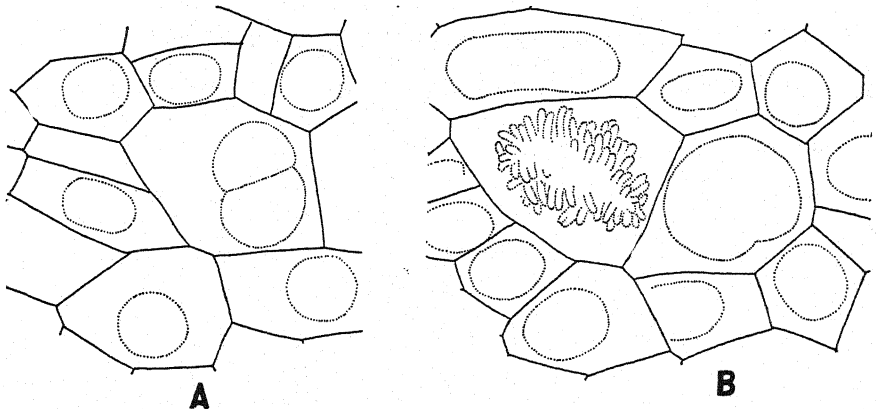
The material examined did not reveal the events in the second division in the few cases in which the first division involves merely a lengthwise division of the univalent chromosomes.



TEXT FIG. 8. *A*, group of coalescing pollen mother cells.  $\times 650$ . *B*, large protoplasmic mass of coalesced pollen mother cells. Portions of three small and three large spindles in section. Each large spindle includes the chromosomes from several contiguous pollen-mother-cell nuclei.  $\times 1250$ . *C*, giant "pollen grain" resulting from more or less complete coalescence of the pollen mother cells of the locale.  $\times 147$ .

A very uncommon procedure in sporogenesis is sometimes observed in parts of locules or in entire locules. The cytoplasm of adjacent pollen mother cells, in consequence of feeble wall- or boundary-formation, begins to coalesce, the result being a large, multinucleate mass of protoplasm (text fig. 8 *A*.) Two or more nuclei often are in contact, but the nuclear membranes remain intact. The chromosomes of each nucleus may become oriented on an independent spindle, or the chromosomes of contiguous nuclei may become distributed over a single giant spindle (text fig. 8 *B*). In the latter case a single spindle area is probably developed around two or more of the adjoining nuclei, and as the nuclear membranes disappear in the late prophases there remains no barrier between the chromosomes of the individual nuclei. The material examined indicates no synapsis or pairing of chromosomes. The individual unpaired chromosomes move to the poles at random, or, as happens in a small number of locules, divide longitudinally, so that the daughter nuclei may be composed of entire univalent chromosomes or halves of univalent chromosomes. In either case some form of giant "pollen grain" containing small and large nuclei results (text fig. 8 *C*). The smaller nuclei are due to straying chromosomes, and to the failure of the larger chromosome groups to hold together. Pollen mother cells containing 42 unpaired chromosomes are occasionally found. These probably have the same origin as the giant protoplasmic masses just described.

The pollen grains of the haploid sporophyte may vary greatly both as to size and as to the number of nuclei they contain.



TEXT FIG. 9. Somatic tissue from lower half of ovary.  $\times 1250$ . *A*, two nuclei about to fuse. *B*, three large nuclei formed by the fusion in pairs of somatic nuclei. One of the large nuclei is in progress of mitosis.

The type of mitosis tends to be uniform within larger regions of a locule, within an entire locule, or even within an entire anther; hence one anther may contain tetrads comparatively normal in appearance while another may contain tetrads which are conspicuously abnormal.

The nuclei of the somatic cells of the stamen and pistil average smaller than in diploid plants and contain only 21 chromosomes. Fusion of somatic nuclei in the lower half of the ovary (text fig. 9 A) and around the fibrovascular bundles of the anther occurs with conspicuous frequency as compared with diploid plants. These fusion nuclei on division give rise to large mitotic figures (text fig. 9 B). The history of the fusing nuclei has not been determined, but the large size of the cell and the occurrence of binucleate cells suggest a failure in cell-wall formation between daughter nuclei during the rapid growth in these organs. The patches with large nuclei in the ovary consist of only a few to ten or more cells. They appear more frequently near the periphery of the bundles, but occur also between the bundles. Rarely giant nuclei are found. These show evidence of containing four and possibly, sometimes, three sets of chromosomes.

#### DISCUSSION

It is impossible to ascertain what condition in the ovule brought about the haploid state of the sporophyte. The 21 chromosomes are undoubtedly those of *Triticum*, the female parent. If the chromatin from the *Aegilops* pollen entered the egg, it was excluded in the ensuing divisions in the development of the embryo. Injury to the flower during the artificial pollination, or the presence of foreign protoplasm, may have initiated the development of the unfertilized egg.

The presence of only one set of chromosomes evidently produced only slight or no disturbance in the vegetative tissues of the plant. The only observed exception is a decidedly more frequent fusion of nuclei in the somatic cells of the ovaries and anthers. If this deviation in the behavior of the somatic nuclei should take place in the early primordial stage of the flower, it would probably alter the nuclear state of the sporogenous tissue and consequently the course of the ensuing sporogenesis.

The radical disturbance of meiosis in the ovules and anthers parallels that often observed in  $F_1$  plants of some wide crosses. The absence of synaptic mates is evidently as fatal to orderly sporogenesis as is the presence of inharmonious chromosomes in hybrid offspring. The giant multinucleate protoplasmic masses sometimes occurring in the anthers of the haploid plant involved two or more complete sets of chromosomes and hence synaptic mates. The failure to pair in this case is probably due to the tardy convening of the chromosomes, as normal synapsis occurs in the early prophase if not before. If the nuclei had fused early in the development of the spore mother cells, pairing would probably have occurred.

If wheats containing 42 sporophytic chromosomes may be assumed to be hexaploid and to have evolved from wheats with lower chromosome numbers through incomplete mitosis, non-reduction, polyspermy, or any method implying longitudinal division or a duplication of chromosomes, the resulting gametes would be expected to contain pairs of homologous chromo-

somes. If this supposition is correct, the spore mother cells of the haploid Hybrid 128 should contain chromosomes which are more or less closely homologous and pairing might be expected at sporogenesis. It is possible that chromosomes which are homologous when the species is young may become progressively less so. It may also be possible that the orientation or architecture of the chromatin is of such a nature as to preclude pairing unless a new set of chromatin has recently been added, as in fertilization. Then, also, sex differentiation may play a part. However, Collins and Mann<sup>5</sup> report that in crosses between *Crepis setosa* Hall with 4, and *C. biennis* L. with 20, chromosomes (haploid numbers) the F<sub>1</sub> sporogenesis shows 10 bivalents; the 20 chromosomes of *C. biennis* pair with each other, while the 4 chromosomes of *C. setosa* remain unpaired and are distributed in varying numbers to opposite poles. Belling and Blakeslee<sup>6</sup> report no pairing in the haploid plants of *Datura stramonium*, but that each unpaired chromosome moves, apparently by chance, to one or the other pole. The haploid plants of *Datura* possess 12 chromosomes, which number may perhaps be considered primal or basic for *Datura stramonium*. Other forms described by Belling and Blakeslee are characterized by chromosome numbers in multiples of this number. Hybrid 128 in its haploid state is triploid in respect to the genus *Triticum*, the basic number of *Triticum* being 7.

It seems significant in this connection that when *Triticum monococcum* L. (var. *Hornemannii* Kcke.) with 7 chromosomes is crossed with *T. turgidum* L. (var. *pseudo-cervinum* Kcke.) characterized by 14 chromosomes, all the 7 chromosomes of *T. monococcum* find synaptic mates. When *T. vulgare* (or other wheat with 21 chromosomes) is crossed with *T. turgidum* (or other wheat with 14 chromosomes), all the 14 chromosomes of *T. turgidum* find synaptic mates (Sax).<sup>7</sup> When *Triticum vulgare* is crossed with *Aegilops cylindrica*, with 14 chromosomes, only 7 of the *Aegilops* chromosomes find synaptic mates (text fig. 4 B; also Sax and Sax<sup>8</sup>). When *Aegilops* is crossed with *T. turgidum* L. (var. *Plinianum* Kcke.), none of the *Aegilops* chromosomes find synaptic mates (research in progress). These facts suggest the hypothesis illustrated by the diagrams in text figure 10. It is here supposed that the gametes of the 21-chromosome wheats contain three dissimilar sets, *a*, *b*, and *c*, of 7 chromosomes each. Two of these sets, *a* and *b*, are duplicated in *T. turgidum*, and the third set, *c*, is duplicated in *Aegilops cylindrica*, but missing in *T. turgidum*. *Aegilops* and *T. turgidum*

<sup>5</sup> Collins, J. L., and Mann, Margaret C. Interspecific hybrids in *Crepis* II. *Genetics* 8: 212-232. 9 text figs. 1923.

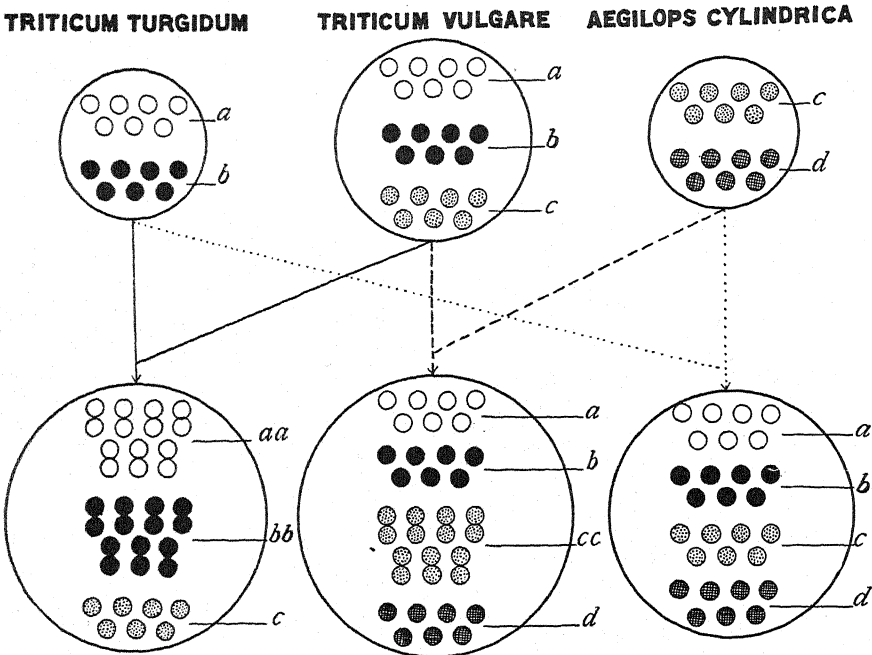
<sup>6</sup> Belling, J., and Blakeslee, A. F. The reduction division in haploid, diploid, triploid and tetraploid *Daturas*. *Proc. Nat. Acad. Sci.* 9: 106-111. 1923.

<sup>7</sup> Sax, K. Sterility in wheat hybrids II. Chromosome behavior in partially sterile hybrids. *Genetics* 7: 513-552. 3 Pl. 1922.

<sup>8</sup> Sax, K., and Sax, Hally J. Chromosome behavior in a genus cross. *Genetics* 9: 454-464. 2 Pl. 1924.



have no sets in common. Thus when a 21-chromosome wheat is crossed with *T. turgidum*, the chromosomes in sets *a* and *b* pair respectively, but the chromosomes in set *c*, finding no synaptic mates, remain unpaired. When the 21-chromosome wheat is crossed with *Aegilops*, the chromosomes in



TEXT FIG. 10. Diagram illustrating hypothetical relationships of chromosomes. The 7 chromosomes in set *a* and the 7 chromosomes in set *b* are present in both *Triticum vulgare* (21 chromosomes as the haploid number) and in *T. turgidum* (14 chromosomes). The 7 chromosomes in set *c* are present in *T. vulgare* and in *Aegilops cylindrica* but not in *Triticum turgidum*. The 7 chromosomes in set *d* are present in *Aegilops* but not in either *T. vulgare* or *T. turgidum*. A 21-chromosome wheat  $\times$  a 14-chromosome wheat gives rise to sporocytes with 14 paired and 7 unpaired chromosomes (lower left). A 21-chromosome wheat  $\times$  *Aegilops cylindrica* gives rise to sporocytes with 7 paired and 21 unpaired chromosomes (lower center). *Aegilops cylindrica*  $\times$  *Triticum turgidum* gives rise to sporocytes with 28 unpaired chromosomes (lower right).

set *c* pair and chromosomes in sets *a* and *b*, lacking synaptic mates, remain unpaired, and for the same reason the chromosomes in set *d* of *Aegilops* remain unpaired. When *Aegilops* is crossed with *T. turgidum*, all the four sets of chromosomes are dissimilar, and hence no homologous mates are present and pairing can not take place. The failure of chromosome pairing during the sporogenesis of haploid Hybrid 128 is due to the absence of homologous mates.

## SUMMARY

1. Pollination of Hybrid 128, *Triticum compactum humboldtii*, with pollen of *Aegilops cylindrica* resulted in a plant like the female parent in appearance, but almost completely sterile, producing nine seeds, or one seed per 380 flowers.
2. The nuclei of the spore mother cells and of the somatic cells of the stamens and pistils contain 21 single chromosomes.
3. Sporogenesis is irregular, as in some wide crosses. The 21 unpaired chromosomes are distributed in the first division, apparently by chance, to the two poles. Chromosomes frequently stray in the cytoplasm.
4. The pollen grains vary greatly as to size and as to the number of nuclei they contain.
5. Giant multinucleate "pollen grains" are sometimes found as a result of the coalescence of contiguous pollen mother cells.
6. Fusion of somatic nuclei in pairs, and subsequent mitosis, frequently occur in ovaries and anthers.
7. The haploid Hybrid 128 is triploid in respect to the genus *Triticum*, and its nuclei probably contain three dissimilar sets of seven chromosomes each. Synapsis fails, possibly on account of the absence of homologous chromosomes.
8. The absence of homologous mates seems to be as fatal to orderly meiosis as the presence of inharmonious chromosomes in hybrid offspring.

# SECOND REPORT ON THE USE OF CHEMICALS FOR HASTENING THE SPROUTING OF DORMANT POTATO TUBERS<sup>1</sup>

F. E. DENNY

(Received for publication April 9, 1926)

## INTRODUCTION

In a previous paper (1) it was shown that a number of different chemical treatments were effective in breaking the rest period of Irish potatoes (*Solanum tuberosum* L.). In the autumn and winter of 1925-1926 a large number of additional tests were carried out, using the same chemicals and also others not previously tried. This paper gives the results of these later tests, and offers suggestions as to the most promising methods of treatment based on the experience so far obtained with the different varieties.

## VARIETIES USED

The Bliss Triumph potatoes were from the Maine 1925 crop and were shipped by express at once after digging. They were fully mature but dormant. Untreated tubers planted October 15 showed general sprouting above ground about December 5.

The Irish Cobbler potatoes were from the late crop in New Jersey. They were harvested October 26 from vines that were still green when caught by frost. They were therefore immature when dug and were very dormant. General sprouting of untreated tubers did not occur until February 15, 1926. Since this lot of Irish Cobblers was immature when dug, a test was made using the same treatment at three different dates, namely, 10 days, 22 days, and 36 days after digging. The results obtained depended partly upon the time after digging at which the treatment was applied.

Potatoes of the Green Mountain variety were obtained from the late crop in Western Maryland. They were shipped by express to Yonkers, and treatments were started within about one week after the tubers had been removed from the soil. These tubers were not very dormant, untreated tubers showing good sprouting in about two months after planting.

The McCormick potatoes were grown in the Institute gardens as a late crop, were harvested October 15, and stored at room temperature. Experimental work with McCormick was not begun until November 10. In considering the results with McCormick, therefore, it should be noted that

<sup>1</sup> Published, at the expense of the Boyce Thompson Institute for Plant Research, out of the order determined by the date of receipt of the manuscript.

the tubers were not treated until nearly a month after digging. They were deeply dormant, however, the untreated tubers not showing general sprouting until February 15, 1926.

#### METHODS

Three methods of treating the tubers were used. They will be referred to in the following pages as the "dip method," "vapor method," and "soak method." The general procedure with each method (omitting, for the present, consideration of the particular concentration of chemical and time of treatment) was as follows:

*Dip Method:* The tubers were cut into suitable size for planting (about 1 oz.), were dipped into a solution made by dissolving a certain amount of chemical in water, were removed at once from the dipping solution, and were placed in a closed container that had a volume not more than about twice the volume of the sample, and were stored in the container for a certain length of time. This method could be used with substances like ethylene chlorhydrin, which give off a vapor on standing. By this procedure a uniform distribution of liquid over the surface of the potato was obtained. The vapors penetrated the tissue and brought about changes which resulted in early sprouting. The concentrations and period of storage will be discussed when consideration is given to the results with the different varieties.

*Vapor Method:* Either whole tubers or cut tubers ready for planting were placed in metal containers that could be closed (in ash cans, for example). The desired amount of volatile chemical was placed in shallow dishes that stood on pedestals inserted for the purpose of raising the chemical above the potatoes in order to favor evaporation. The air was not stirred, except in some of the later experiments in which a special gas-tight house of about 150 cubic feet capacity equipped with an electrical fan was used. For large-scale treatments it would probably be necessary to stir the air with fans in order to obtain an even distribution of the vapors which, in general, are heavier than air.

*Soak Method:* The cut tubers were soaked in solutions of different chemicals of varying concentrations for periods of time varying from one to two hours. After the period of treatment the treated pieces were rinsed in tap water and planted. The soak method has two serious disadvantages: (1) the trouble involved in handling potatoes when the treated material must remain submersed for an hour or two, and (2) the diluting effect on the soaking solution that results from successive treatments in the same solution. Nevertheless, this method gave good results especially when thiocyanate was used with Irish Cobbler.

## EXPERIMENTAL

Results with Ethylene Chlorhydrin ( $\text{C}_2\text{H}_4\text{Cl}_2$ )<sup>2</sup>

*Dip Method:* The dip method, using a dipping solution containing concentrations of ethylene chlorhydrin varying from 15 cc. to 45 cc. per liter of water and with subsequent storage periods varying from 16 to 24 hours, or with a dipping solution of 5 to 20 cc. per liter with a storage period of 48 hours, gave notably good results with Bliss Triumph. Photographs of these results are shown in Plate XXXI, figures 1 and 2, and Plate XXXII, figures 4 and 5. It is apparent from the photographs that there is a considerable range of concentration of dipping solution and of time of subsequent storage over which forcing effects can be obtained; but it is also seen that within this range a maximum effect is attainable. No doubt the conditions necessary for a maximum will be found to vary with the stage of dormancy and possibly also with the crop from different localities or with the harvest of different years. Whether it is possible to find a treatment that will permit of the use of the most favorable concentration and time of treatment under any given set of conditions can be determined only by further experiments.

The dip method also gave good results with the Green Mountain variety, using the same concentrations and same lengths of exposure as those shown above for Bliss Triumph. In the case of the McCormick variety, cut tubers, the dip method, using 30 cc. and 45 cc. per liter, and storing 16 hours, gave good stimulation as is shown in Plate XXXII, figure 6, lots *B* and *C*. This appeared to be the minimum strength, however, and it may be that the storage period should be increased for McCormick. Cut tubers of the Irish Cobbler variety when treated 10 days after digging did not respond to the dip method at these concentrations and for storage periods up to 24 hours; better results were obtained by treating 22 days after digging, but it was not until 36 days after digging that good forcing effects were obtained. Further experiments will be carried out, using longer storage periods after dipping.

*Vapor Method:* The vapor method, using cut tubers of the Bliss Triumph variety, gave good forcing action at a concentration of 0.5 cc. per liter of air space for 24 hours and 1.0 cc. per liter of air space for 16 hours; but 1 cc. for 24 hours injured, and 0.25 cc. for 48 hours, although the results were better than those with the check lot, showed less favorable stimulation. The sprouting of Green Mountain tubers was hastened by 0.5 cc. ethylene chlorhydrin per liter of air space for 16 hours, and the same concentration for 24 hours was satisfactory for McCormick. In the case of the Irish Cobbler variety, the vapor method, using 0.5 cc. per liter of air space for 16 hours, hastened sprouting at the 22-day period, but was ineffective at the 10-day period and caused some injury at the 36-day period after digging.

<sup>2</sup> The ethylene chlorhydrin referred to in this paper is the commercial 40-percent solution, not the anhydrous chemical.

One experiment was carried out with Bliss Triumph, using the vapor method with whole tubers and planting the intact tuber without cutting it into pieces. In this case 2 cc. of ethylene chlorhydrin per liter of air space were used, the period of treatment being 24 hours. Favorable results were obtained, as are shown in Plate XXXIII, figure 7. In considering this photograph it should be noted that, while the check tubers sent out one sprout per tuber, the treated potatoes sent out several sprouts per tuber, all sprouts being located at the apical end. The sprouts were vigorous in spite of their number.

When whole tubers were treated by the vapor method and planted cut, the result obtained depended upon whether the tubers were cut and planted at once or were stored in air for various periods before cutting and planting. It was found that storage after treatment, before planting, avoided toxicity and that the favorable effects of the treatment in hastening germination were not lost by delaying the time after treatment at which the tubers were cut into pieces and planted in the soil. This phase of the work is discussed in another section of this paper (p. 391).

*Soak Method:* Experiments with Bliss Triumph showed that soaking one hour in a solution containing 4 to 6 cc. of ethylene chlorhydrin per liter of water gave good forcing action. However, when results can be obtained by either the dip method or the vapor method (as is the case with Bliss Triumph) the soak method is not recommended. In the case of McCormick potatoes, 4 cc. per liter and soaking 2 hours gave hastened germination (Pl. XXXIII, fig. 9, lot U), and 6 cc. per liter forced the Irish Cobblers even at the 10-day period after digging. This treatment was also satisfactory at the 22-day period but caused injury at the 36-day period.

#### Results with Solutions of Sodium and Ammonium Thiocyanate (NaCNS), (NH<sub>4</sub>CNS)

*Bliss Triumph:* Soaking cut tubers one hour in 2-percent sodium thiocyanate caused a little injury but forced early sprouting of those not injured. One-percent sodium thiocyanate for 1 hour or for 2 hours was favorable, and sprouting considerably better than that of the untreated lots was obtained with 0.5 percent for 2 hours. In a subsequent experiment, using Bliss Triumph from Bermuda, the results with ammonium thiocyanate were better than with sodium thiocyanate, good results being obtained with 3-percent, 2-percent, or 1-percent ammonium thiocyanate for 1 hour. The margin between stimulative and injurious concentrations has not been large in the case of sodium or potassium thiocyanate treatments with Bliss Triumph. It may be that ammonium thiocyanate will give a wider range. Where a reduction in the percentage germination would not be serious, for example, in forcing early germination of tubers for experiments in plant pathology, the thiocyanate treatment could be used with confidence; but for general planting, where a full stand is wanted, the thiocyanate treatment has not yet given a good margin of safety for Bliss Triumph.

*Irish Cobbler*: In the case of Irish Cobbler, however, the thiocyanate treatment has been uniformly successful. The soak method, using 2-percent sodium thiocyanate for 1 hour, was notably successful with this lot of Irish Cobblers at all three periods of treatments. Four-percent sodium thiocyanate broke the dormancy at the 10-day period, but caused injury at the 22-day period. One-percent sodium thiocyanate was partly effective at the 10-day period after digging and gave excellent stimulation at the 22- and 36-day periods. Plate XXXIII, figure 8, shows the results obtained with sodium thiocyanate.

*Green Mountain*: Early sprouting of this variety was forced by soaking 1 hour in 2-percent sodium thiocyanate.

*McCormick*: Soaking cut tubers for 1 hour in 2-percent sodium thiocyanate and for 2 hours in 1-percent sodium thiocyanate gave good sprouting, as is shown in Plate XXXIII, figure 9, lots *T* and *V*. Four-percent sodium thiocyanate for 1 hour was too strong.

### Results with Ethylene Dichlorid ( $C_2H_4Cl_2$ )

*Bliss Triumph*: Ethylene dichlorid, a chemical first recommended by McCallum (2), gave promising results. Cut tubers responded well to a 24-hour treatment with a concentration equal to 0.5 to 1.0 cc. of ethylene dichlorid in 17.5 liters of air space (equal to 0.028 to 0.057 cc. per liter of air). An injurious concentration for Bliss Triumph was reached at about 2.0 cc. for a 17.5-liter space (about 0.1 cc. ethylene dichlorid per liter of air).

*McCormick*: A favorable response of the McCormick variety to vapors of ethylene dichlorid was obtained, as is shown in Plate XXXI, figure 3, lots *J*, *K*, and *L*. The effective concentration ranged from 2.0 cc. to 0.5 cc. in 17.5 liters of space (from 0.1 to 0.028 cc. per liter of air). For whole-tuber treatments, 4 cc. in 17.5 liters of air (0.2 cc. ethylene dichlorid per liter of air) for 24 hours caused early sprouting. Half the concentration was noticeably less effective. It will be noted that relatively low concentrations of ethylene dichlorid were effective. Because of its cheapness and availability as a standard commercial product, it is hoped that a favorable procedure can be worked out.

### Results with Ethyl Bromid ( $C_2H_5Br$ )

Ethyl bromid, another chemical suggested by McCallum (2), forced the sprouting of Bliss Triumph, cut tubers, by a 48-hour treatment, using 0.5 cc. for each 17.5 liters of air space (0.028 cc. per liter of air), and fairly well by a 24-hour treatment, using 1.0 cc. for each 17.5 liters of air space.

In the case of the McCormick variety, ethyl bromid was successfully used with cut tubers at a concentration of 3 cc. in a 17.5-liter space for 16 hours (see Pl. XXXI, fig. 3, lot *M*). With whole tubers specially favorable results were obtained with 4 cc. in a 17.5-liter space for 24 hours.

### Results with Thiourea ( $\text{NH}_2\text{CSNH}_2$ )

Soaking cut tubers in 2- to 4-percent thiourea solutions broke the dormant period of Bliss Triumph, Irish Cobbler, and McCormick varieties. Several derivatives of thiourea were tested, and one of these, o-tolylthiourea, gave good results, a saturated solution of this chemical causing early germination after 1 hour's soaking.

Thiourea also caused the development of more than one sprout per eye, and in some cases more than one eye per seed-piece. This effect upon bud-inhibition and apical dominance will be considered more fully in another paper (Bot. Gaz., in press).

### Results with Other Chemicals

Ethyl iodid,  $\text{C}_2\text{H}_5\text{I}$ , a substance not heretofore used for breaking the dormancy of potatoes but tested by Stuart (3) on dormant bulbs and shrubs, was effective with cut tubers of the Bliss Triumph variety in unusually low concentrations,  $\frac{1}{6}$  cc. in a 17.5-liter space for 24 hours giving good sprouting (equal to 0.0095 cc. per liter of air).

Xylol,  $\text{C}_6\text{H}_4(\text{CH}_3)_2$ , hastened sprouting fairly well when 1 cc. in a 17.5-liter space was applied to cut tubers for 24 hours. The range with carbon tetrachlorid,  $\text{CCl}_4$ , was very narrow, 2 cc. in a 17.5-liter space being too high and 0.5 cc. being too low. When 1.0 cc. was used the results were satisfactory. Dichloroethylene,  $\text{C}_2\text{H}_2\text{Cl}_2$ , trichloroethylene,  $\text{C}_2\text{HCl}_3$ , and carbon bisulphid,  $\text{CS}_2$ , gave results similar to those reported in the previous paper (1).

Ethylene,  $\text{C}_2\text{H}_4$ , propylene,  $\text{C}_3\text{H}_6$ , and acetylene,  $\text{C}_2\text{H}_2$ , were tried with both whole and cut tubers at concentrations of 1 to 100 and 1 to 1,000 of air for 4 days and 7 days. In no case were favorable forcing effects noted. Methyl chlorid gas at a concentration of 1 part methyl chlorid to 100 parts of air, for 4 days, gave considerably better responses. The concentration and time of treatment required for this gas, however, make its use impractical.

Ethylene dibromid,  $\text{C}_2\text{H}_4\text{Br}_2$ , was notable, not for its forcing effects but because of its high toxicity. Even 0.01 cc. in 17.5 liters of air space injured cut tubers of Bliss Triumph with a 24-hour treatment. This is equivalent to about 0.0006 cc. of ethylene dibromid per liter of air space. The stimulative effects of this chemical, however, at any concentration tried were slight or negligible.

### Effect of Storage in Air after Treatment on the Toxicity of Chemicals and upon Subsequent Sprouting of Tubers

When it was found that whole tubers could be successfully treated with vapors, the question at once arose, Must the treated tuber be planted at once, or can it remain in storage until a later planting time without losing the benefit of the treatment? It was found not only that storage subsequent to treatment was possible, but that the toxic effects of the vapor were wholly or at least partly avoided.



Thus, Bliss Triumph whole tubers were treated with concentrations of ethylene chlorhydrin varying from 6 cc. per liter of air space for 24 hours to 1 cc. for 48 hours. A few tubers were removed at once, were cut into pieces, and planted. The remainder of the treated tubers were stored in air in paper bags, and a sample was removed at intervals of one week and planted after being cut into pieces in the usual way. The tubers that were cut and planted at once after treatment rotted, but those planted after subsequent storage in air did not rot but sprouted much sooner than check tubers, handled in the same way but receiving no chemical treatment. Thus, as appears in Plate XXXIV, fig. 11, the treated lot after storage in air for 7 days showed the favorable effect of the chemical treatment, and in Plate XXXIV, figure 12, it is seen that the forcing effect of ethylene chlorhydrin is not lost by storage in air for 21 days after treatment. In fact, in the experiment, the treated tubers began to sprout in air before they were planted, which fact indicates that the growth processes that are started by the treatment continue in action without requiring the favorable influence of a moist soil.

The same effect to a less marked extent was noted with Irish Cobbler. Whole tubers treated with 1 cc. of ethylene chlorhydrin for 24 hours when cut into pieces and planted at once after treatment rotted. But when stored 7 days in air after treatment before cutting and planting, 9 out of 14 pieces lived and sprouted, and after storage for 14 days 13 out of 14 pieces lived and sprouted.

In the case of the Green Mountain variety, 4 cc. of ethylene chlorhydrin per liter of air for 24 hours killed the tubers if they were cut and planted at once after treatment; but, if the whole tubers were allowed to stand in air for 4 days, prompt and complete germination resulted. When the concentration used was 1 cc. per liter for 24 hours, 50 percent loss resulted when the tubers were cut and planted at once; but only 1 day's standing in air before planting was required to prevent this toxicity and at the same time to hasten the sprouting of the buds.

The fact that potatoes may be treated previous to the time at which they are to be planted is important from a practical standpoint, for it indicates the possibility that the tuber treatments could be made in large quantities by firms that grow potatoes for seed. The treated potatoes could then be shipped to purchasers and would arrive ready for immediate planting. Additional experiments are needed before such a procedure can be recommended. It must be shown, first of all, that tubers of various varieties can be successfully treated at once after removal from the soil, and secondly, that uniform results with the crops from different soils and in different years can be obtained.

### Results in Special "Gassing-house" under Semi-commercial Conditions

A special gas-tight room with a volume of 150 cubic feet was constructed; it was equipped with an electric fan for stirring the air, and had removable shelves with wire-screen bottoms for holding the potatoes.<sup>3</sup> From preliminary experiments carried out, using this room, it is likely that a smaller amount of ethylene chlorhydrin can be used under these conditions than was found necessary when the air was not stirred. Thus, McCormick whole tubers responded to a concentration of 0.35 cc. per liter of air space for a 20-hour treatment (see Pl. XXXIV, fig. 10). Much larger amounts were required under conditions in which the air was not stirred. This result is probably due to the fact that a more complete evaporation of the ethylene chlorhydrin was obtained by stirring the air. Since the ethylene chlorhydrin is a 40-percent solution in water, the vapors are not completely removed from the water unless the air is stirred.

### Comparison with Results of Previous Experiments

In general the results previously reported were confirmed, the same chemicals giving good forcing action on dormant tubers. The principal differences between the results of the first series of experiments and those reported in this paper are: (1) The margin of concentration for the soak method with Bliss Triumph was given as 3 to 10 cc. of ethylene chlorhydrin per liter of water. In the second series not more than 6 cc. per liter could be used without injury. (2) The ethylene chlorhydrin treatments were less favorable with Irish Cobbler in the second series. Irish Cobbler potatoes treated at once after removal from the soil did not respond satisfactorily to this treatment; after a storage period of about 22-36 days the treatments showed good results. It may be that the immaturity of this lot of Irish Cobblers as compared with those of the 1925 experiments may account for the difference. Further experiments on this point will be carried out.

### RECOMMENDED TREATMENTS

I list below the treatments that have been found successful with different varieties in these tests. Since the problem is still in the experimental stage, and since potatoes grown in different localities and in different stages of dormancy may respond differently, a range of concentrations and times of treatment is given in order that other experimenters may more easily determine the most favorable treatment for their special conditions.

*For Bliss Triumph:* Ethylene chlorhydrin, dip method, cut tubers, 30 cc. per liter of water, storage time 24 hours; or 40 cc. for 16 hours; or 10 cc. for 48 hours; ethylene chlorhydrin, vapor method, cut tubers, 0.5 cc. per liter of air space for 24 hours; or 1 cc. per liter of air space for 16 hours. Ethylene chlorhydrin, whole tubers, 1 cc. per liter of air space, for

<sup>3</sup> I am indebted to Mr. William Stuart of the United States Department of Agriculture for suggestions regarding the construction of this room.

24 hours; let the whole tuber stand in air for one or two days after treatment, then cut into pieces and plant. Ammonium, sodium, or potassium thiocyanate, 2-percent solution, soak for 1 hour; also 3-percent for 1 hour, or 1-percent for 2 hours; the tubers must be dormant for this treatment, otherwise injury will be likely to be produced.

*For Irish Cobbler:* Ammonium, sodium, or potassium thiocyanate, cut tubers, 2-percent solution, soak for 1 hour; also 3-percent for 1 hour, or 1-percent for 2 hours. Ethylene chlorhydrin, cut tubers, soak method, 6 cc. per liter of water, 1 hour; also 4 cc. per liter of water for 1 hour or for 2 hours. With tubers that were mature at the time of harvest the following treatment gave good results: ethylene chlorhydrin, vapor method, whole tubers, 0.5 cc. per liter of air space for 24 hours, tubers cut into pieces after treatment and planted.

*For Green Mountain:* Same as for Bliss Triumph.

*For McCormick:* The recommendations are based upon treatments applied to tubers that had been stored about a month in air after digging. Ethylene chlorhydrin, dip method, 45 cc. per liter of water, store 24 hours; or 30 cc., store 24 hours; or 20 cc., store 48 hours. Ethylene chlorhydrin, vapor method, cut tubers, 0.5 cc. per liter of air space for 24 hours; or 1.0 cc. for 16 hours, or 0.25 cc. for 24 hours. Ammonium, sodium, or potassium thiocyanate, cut tubers, 1 hour in 2- or 3-percent solution; or 1 hour or 2 hours in 1-percent solution. Ethylene dichlorid, cut tubers, 0.06 cc. per liter of air space for 16 hours; also 0.1 cc. or 0.03 cc. for 16 hours. Ethylene dichlorid, whole tubers, 0.2 cc. per liter of air space for 24 hours.

#### SUMMARY

1. In general, the results of former experiments were confirmed. Ethylene chlorhydrin, sodium thiocyanate, ethylene dichlorid, ethyl bromid, carbon bisulphid, and trichloroethylene forced early sprouting of dormant potatoes.

2. The principal difference found in the second series of experiments as compared with the first was that ethylene chlorhydrin was less effective against Irish Cobbler in the early stages of dormancy. This difference may be due to the immaturity of the tubers of this lot at the time of digging.

3. The following chemicals not mentioned in the previous paper were found to be effective in hastening sprouting: ammonium thiocyanate, ethyl iodid, and o-tolyl-thiourea.

4. Four varieties were tested: Bliss Triumph, Irish Cobbler, Green Mountain, and McCormick. Recommendations based on experience obtained in both series of tests are given for forcing the sprouting of tubers of these varieties.

5. It was found that, after whole tubers had been treated by the vapor method, it was not necessary to plant the treated tubers at once. The favorable effects remained in the tuber for at least three weeks after treat-

ment; in fact, the tubers began to sprout in air before being planted in the soil. This indicates the possibility that tubers could be treated in the locality where they are grown, and shipped to a distant locality for planting.

6. Storage of whole tubers in air after treatment before planting also aided in avoiding the toxicity of the chemicals. Tubers treated with certain concentrations of ethylene chlorhydrin, when cut into pieces and planted at once, often rotted, but samples of the same lot held in air a few days after treatment before cutting and planting did not rot, but showed early sprouting with healthy sprouts.

7. The effect of solutions of thiourea in causing the sprouting of multiple buds per eye, and in partially nullifying the dominance of the apical bud, was again noted in treatments with the Bliss Triumph, Irish Cobbler, and McCormick varieties.

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### EXPLANATION OF PLATES

#### PLATE XXXI

FIG. 1. Lot T: Ethylene chlorhydrin, dip method, cut tubers; used dipping solution containing 10 cc. per liter of water; storage period 24 hours. Bliss Triumph. Lot V: Same, but used 20 cc. of ethylene chlorhydrin per liter of water.

FIG. 2. Lot W: Same, but used 40 cc. of ethylene chlorhydrin per liter of water. Lot X: Check, same, but used water instead of ethylene chlorhydrin.

FIG. 3. Lot J: Ethylene dichlorid, vapor method, cut tubers; 2 cc. in 17.5 liters of air space for 16 hours. McCormick. Lot K: Same, but used 1 cc. Lot L: Same, but used 0.5 cc. Lot M: Ethyl bromid, vapor method, cut tubers; 3 cc. in 17.5 liters of air space for 16 hours. McCormick. Lot N: Check, cut tubers stored in air in closed container for 16 hours, then planted. Lot O: Same, but stored 7 days. Lot P: Same, but stored 4 days. *Note:* Lots O and P were checks on a treatment given to other lots not shown in figure 3.

#### PLATE XXXII

FIG. 4. Lot Z: Ethylene chlorhydrin, dip method, cut tubers; used dipping solution containing 5 cc. of ethylene chlorhydrin per liter of water; storage period 48 hours. Bliss Triumph. Lot M: Same, but used 10 cc. of ethylene chlorhydrin per liter of water.

FIG. 5. Lot P: Same, but used 20 cc. of ethylene chlorhydrin per liter of water. Lot Q: Check, same, but used water instead of ethylene chlorhydrin.

FIG. 6. Lot A: Ethylene chlorhydrin, vapor method, cut tubers; 0.5 cc. per liter of air space for 16 hours. McCormick. Lot B: Ethylene chlorhydrin, dip method, cut tubers; dipping solution 45 cc. of ethylene chlorhydrin per liter of water; storage period 16 hours. McCormick. Lot C: Same, but 30 cc. of ethylene chlorhydrin per liter of water. Lots D and E: Checks, not treated. *Note:* Lot W, shown in figure 9, Plate XXXIII, is also a check on lot A shown in this figure.

## PLATE XXXIII

FIG. 7. Lot *E*: Ethylene chlorhydrin, vapor method; tubers treated whole and planted intact; 2 cc. of ethylene chlorhydrin per liter of air space for 24 hours. Bliss Triumph. Lot *M*: Checks, not treated.

FIG. 8. Lot *F*: Sodium thiocyanate, soak method, cut tubers; 4 percent for 1 hour. Irish Cobbler. Lot *G*: Same, but used 2 percent sodium thiocyanate. Lot *H*: Checks, soaked 1 hour in water.

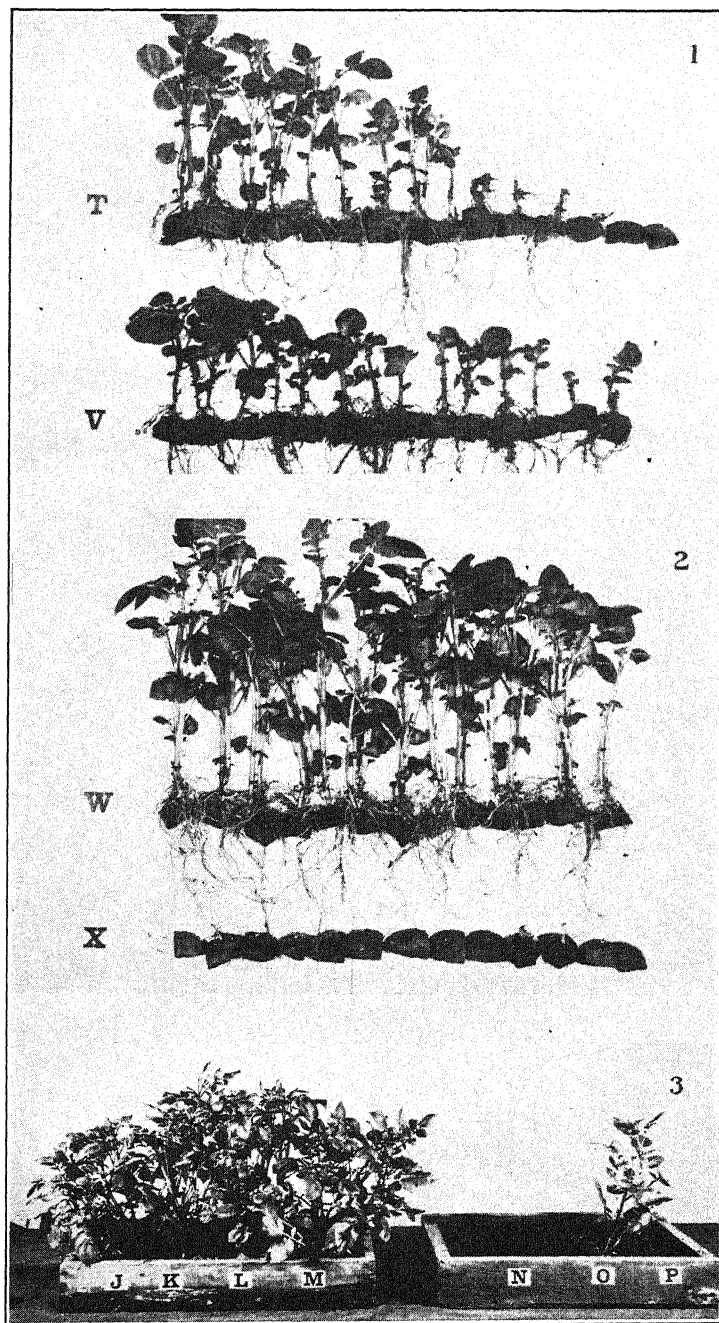
FIG. 9. Lot *T*: Sodium thiocyanate, soak method, cut tubers; 1 percent for 2 hours. McCormick. Lot *V*: Same, but 2 percent, 1 hour. Lot *U*: Ethylene chlorhydrin, soak method, cut tubers; 4 cc. of ethylene chlorhydrin in 1 liter of water; soaked 2 hours. McCormick. Lot *W*: Check, not treated. Lot *X*: Check, soaked 2 hours in water. Lot *Y*: Check, soaked 1 hour in water.

## PLATE XXXIV

FIG. 10. Lot *H*: Ethylene chlorhydrin, vapor method in special "gassing-house," using electric fan for stirring the air; 1,500 cc. of ethylene chlorhydrin in 4,250 liters of air space; treatment for 20 hours; whole tubers treated, allowed to stand in air one day after treatment, then cut and planted. McCormick. Lot *K*: Check, not treated.

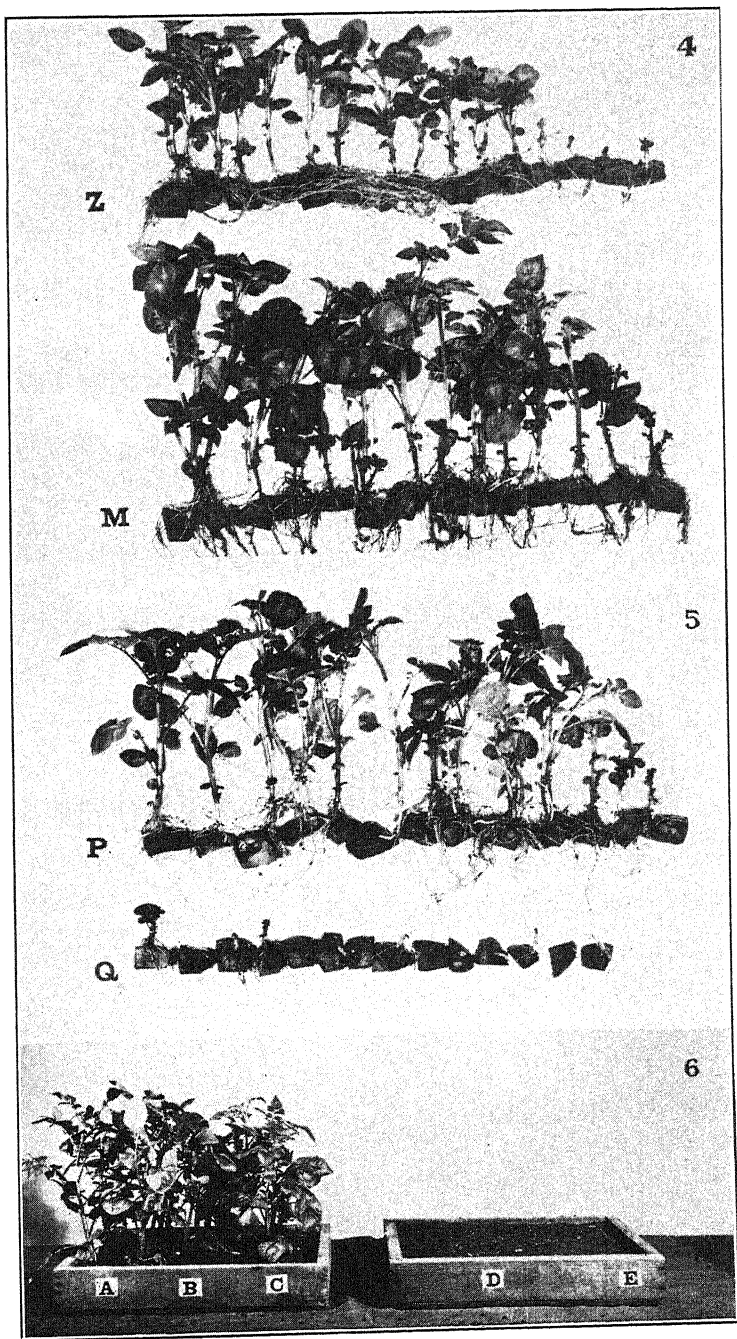
FIG. 11. Lot *C*: Ethylene chlorhydrin, vapor method, whole tubers treated; 1 cc. of ethylene chlorhydrin per liter of air space for 48 hours; tubers allowed to stand in air 7 days, then cut and planted. Bliss Triumph. Lot *Z*: Checks, allowed to stand in air 7 days, then cut and planted.

FIG. 12. Lot *S*: Ethylene chlorhydrin, vapor method, whole tubers treated; 3 cc. of ethylene chlorhydrin per liter of air space for 24 hours; tubers allowed to stand in air 21 days after treatment, then cut and planted. Bliss Triumph. Lot *W*: Checks, allowed to stand in air for 21 days, then cut and planted.



DENNY: SPROUTING OF DORMANT TUBERS





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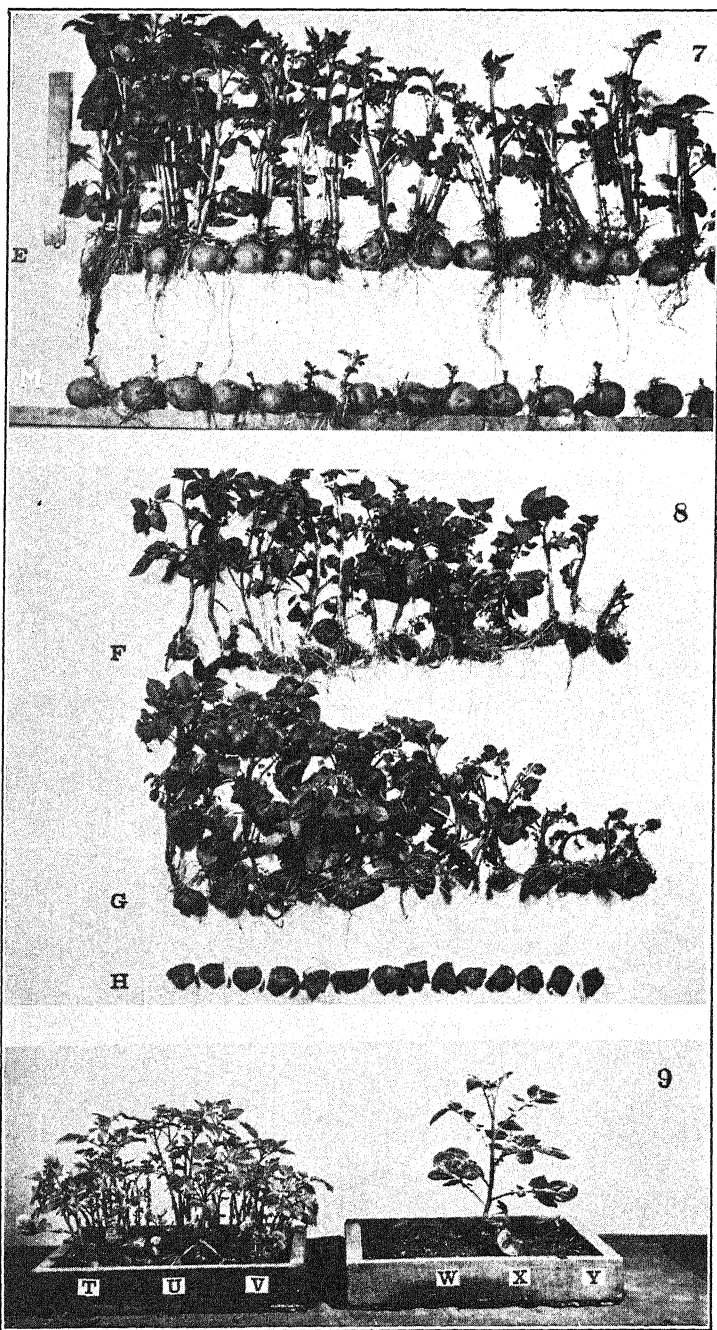
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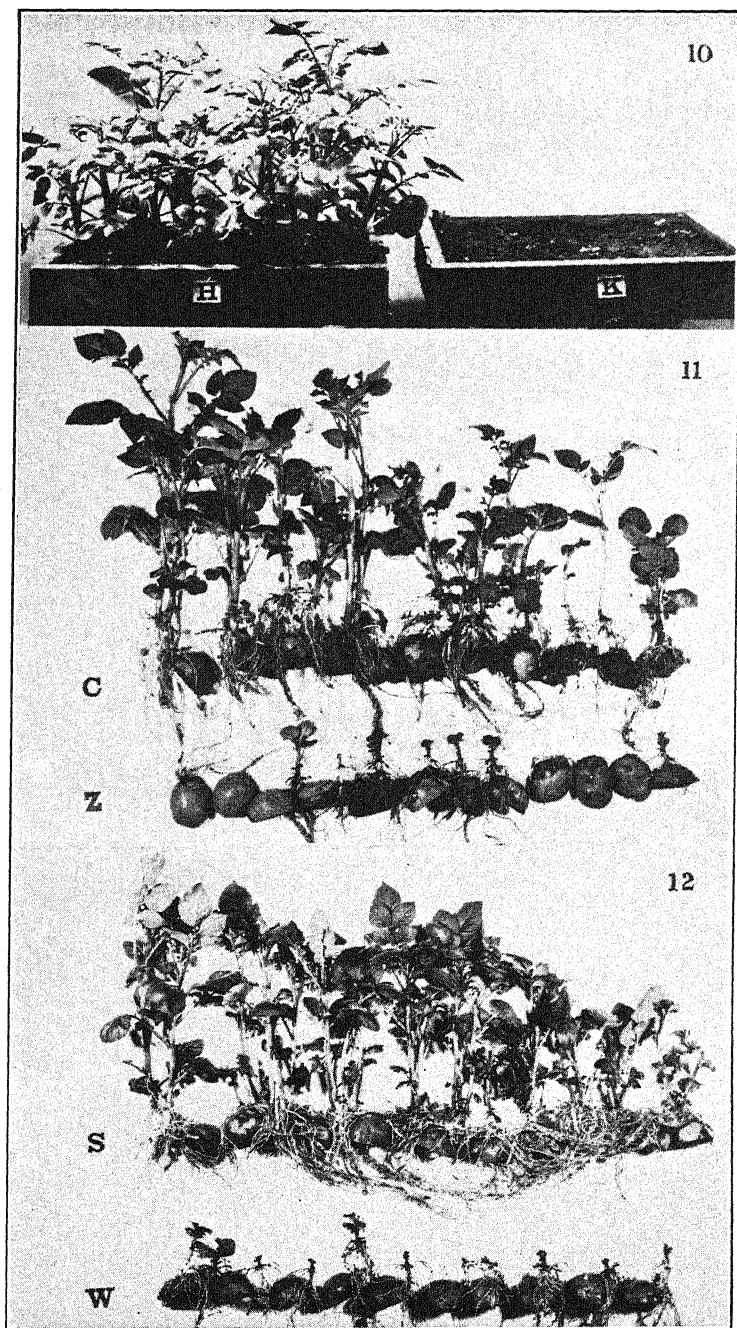
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## FLORAL ANATOMY OF SEVERAL SPECIES OF PLANTAGO

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*Plantago* is one of three genera which, according to Bentham and Hooker (1), make up the family Plantaginaceae. Of the other two, *Bougeria* and *Littorella*, only the latter, a small aquatic genus of one species, is given in Gray's Manual (5) as occurring in the United States. *Plantago* has about two hundred species (de Candolle, 2), scattered over the earth but most abundant in Europe and America. All the species are herbaceous except a few, which are shrubby.

The small order of the Plantaginales, containing only the family Plantaginaceae, is of peculiar interest morphologically, since it shows some characters of a very advanced condition such as sympetaly, reduction in number of parts, and adnation; while at the same time other characters suggestive of primitiveness are present, such as wind-pollination in most species and an aquatic habit in a few.

Little is known of the relationships of the Plantaginales. They are described by some as an anomalous group. Wernham (8) places them as an offshoot from the direct line descended from the Geranial plexus, which came from the common Ranalian ancestor. He believes that the Plantaginales split off much earlier than the Apocynal stock which gave rise to the transitional Tubiflorae (Polemoniales) group, from which were derived, finally, Labiatae and Acanthaceae.

Coulter (3) and Jeffrey (7) have pointed out the importance of the study of vascular anatomy as giving more definite basis for classification than the morphology of vegetative structures, which are subject to great variation due to environmental influences. So far as the author has been able to discover, there has been no work done on the vascular anatomy of the flower of *Plantago*. It was with the hope that some interesting facts might be brought out in regard to the primitiveness of the genus that these studies were undertaken.

### GROSS STRUCTURE OF THE FLOWERS

The flowers of three of the most common species of *Plantago* have been studied in detail, namely, *P. lanceolata* L., *P. major* L., and *P. Rugelii* Dcne.

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These three species are almost universal in their distribution throughout the eastern United States. They are among the most common weeds of lawns and roadsides.

*Plantago lanceolata* has its flowers borne in a dense spike, cylindrical or, in the very short ones, subglobose in shape, and ranging in length from about five millimeters to fifty or more. The flowers are arranged spirally on the axis at an angle of about  $60^{\circ}$ .

Each flower of *P. lanceolata* is subtended by a delicate membranous bract, ovate-lanceolate in shape and very slenderly attenuate at the apex. The bract has a thick midrib containing chlorophyll. The flower is perfectly symmetrical, being constructed on a plan of four. It is about three millimeters in length. The sepals are membranous, broadly ovate, and mucronate at the apex, characterized also, like the bract, by a broad green midrib. On many flowers the ventral sepal has two midribs, each nearly as broad as the one when only one is present; when there are two, the sepal is slightly cleft at the apex, forming two points instead of one. In both bracts and sepals the midrib is pubescent with long, slender silky hairs which, in the closely packed buds on the spike, serve as effective protection for the delicate tissues of the young flowers. The sepals are nearly as long as the corolla tube.

The corolla has its four petals united into a slender urceolate tube, constricted at the throat by downward folds between the lobes, which are ovate-lanceolate and spread almost in salver form at the time the stamens are pushing out. The corolla is membranous and very delicate, transparent, and very light brown in color.

The stamens are alternate with the lobes of the corolla, opposite the sepals, each having a slender two-celled anther, versatile on its very slender filament. The filaments arise from the receptacle, are free from the corolla, the upper parts being bent over toward the pistil, the anthers being inverted so that their basal tips are towards the top of the corolla tube and their abaxial faces towards the pistil. In the flower just ready to open the anthers are pressed closely around the pistil. The anthers are pale yellow in color.

There is a globose two-celled ovary with axial placenta, each cell containing a single ovule. The ovary terminates in a slender style whose surface for some distance below the apex is covered with delicate short hairs. This is the stigmatic surface, and the hairs serve to catch and hold the pollen. The stigma is extruded several days before the stamens push out from the corolla, and is evidently receptive at least a day before the stamens are out.

The opening of the flower is characteristic of the genus. The anthers push out slowly, and gradually spread apart; then, one at a time, they are released and straighten out with a spring. The anthers dehisce by a longitudinal slit, scattering the yellow pollen.

The flowers of *P. lanceolata* are commonly hermaphroditic, though it is

not unusual to find plants all of whose flowers are pistillate in consequence of the abortion of the stamens. In such flowers the stamens are present, but the filaments end in delicate leaf-like structures instead of anthers.

The general features of inflorescence and floral parts are the same in all species studied, but specific differences in the flowers of *P. major* and *P. Rugelii* will be pointed out below.

*Plantago major* is much more robust in appearance than *P. lanceolata*, producing much longer spikes, from one to four decimeters in length. The arrangement of flowers on the spike is spiral, but the spike is less compact than in *P. lanceolata* and the position of the flowers on the axis is more nearly erect, the adaxial, or dorsal, face of the flower being closely pressed against the axis of the inflorescence.

The bracts are very convex, and have a very broad, thick midrib strongly keeled, leaving only a wide margin of the membranous tissue around its edge. The shape is broadly obovate, with an obtuse apex.

The sepals are obovate, with broad green midrib, keeled, and reach almost to the throat of the corolla. The midribs of both sepals and bracts are puberulent, otherwise the perianth is glabrous. The corolla tube is slender-urceolate, but slightly curved upwards towards the axis. This, with the more compact arrangement of the bract, somewhat shortens the flower which is just a little over two millimeters in length. There is the same constriction of the throat as in *P. lanceolata*, but the corolla lobes are sharply reflexed in the freshly opened flower, while in *P. lanceolata* this does not occur until the corolla begins to wither.

The arrangement of the pistil and anthers is the same as already described for *P. lanceolata*, but the anthers in *P. major* are dark purplish brown, or brownish purple, with cream-white pollen. The ovary is two-celled, but differs from that of *P. lanceolata* in having numerous ovules.

*Plantago Rugelii* has a flower quite similar in general appearance to that of *P. major*, although the sepals are so strongly keeled as to appear triangular in cross section, which characteristic makes the flower definitely four-angled. The bract is thick and green except for a narrow membranous margin and is lanceolate in shape, tapering to a slender point.

The sepals are broadly lanceolate, green, except for a membranous margin, and are united at the base, forming a cup about the lower part of the corolla. The corolla, including androecium and gynoecium, is lifted up above the base of the calyx by the elongated receptacle, which forms a short stalk. The corolla is slender-urceolate but very slightly curved, with strongly reflexed, lanceolate lobes, brownish in color.

The stamens are arranged as in other species, but each anther has a short, pointed appendage at the base between the two cells and a short horn-like process extending over the upper end of each cell at the point of dehiscence. The dehiscence is, as in the other species, by a longitudinal split. The anthers are violet in color and the pollen is white. The ovary is two-celled and has several ovules in each cell.



The fruit of all species of *Plantago* is characteristically known as a *pyxis*, being a capsule with circumscissile dehiscence, the only difference between the different species being in the region of dehiscence and in the number of seeds contained. The seeds are only two in *P. lanceolata*, while they vary from six to eighteen in other species.

### HISTOLOGICAL STUDIES

The anatomy of the flowers of several species has been studied with the hope of finding some clue as to the primitiveness of the family, and also to find, if possible, some relationship between the Plantaginaceae and those families usually placed nearest this in modern classifications. The method of such studies which seemed most tangible was that of tracing the vascular supply to the different organs in the flower. It was found to be most practicable to study first those species which could be obtained fresh. It was thought that if any very unusual conditions should be found other species could be studied later. The species selected for study were *P. lanceolata*, *P. major*, and *P. Rugelii*, all of which grow abundantly on the Cornell campus.

### Method of Study

The spikes of flowers were collected and killed in a 1 percent solution of chrom-acetic acid, then washed for 24 hours in running water. They were dehydrated in a series of alcohols, using 30 percent, 50 percent, 70 percent, 95 percent, and 100 percent, since the study was only for anatomical features. They were then imbedded in paraffin in the usual way, cut 10 microns in thickness, and mounted with gelatin fixative. They were stained first in 1 percent crystal violet for ten minutes, washed in 95 percent and 100 percent alcohol, then the surplus stain was washed out in erythrosin (0.025 percent) in clove oil. This gave a clear blue color to the xylem, a paler blue to cutinized parts, pale violet to phloem, and pink to the other tissues. In the upper part of the ovary the xylem was so slightly lignified that it did not stain well, but in most cases it could nevertheless be clearly distinguished from the surrounding parenchyma.

A series of cross sections of the spike was made in order to trace the general vascular supply of the flower. Longitudinal sections also were made for the same purpose. A third series was made of cross sections of the flower, beginning at the base of the short flower stalk, in order to trace the vascular supply of all its different organs.

### *Plantago lanceolata*

The origin of the vascular bundle which enters the flower is shown in figure 1, Plate XXXV. There is a large vascular bundle which leaves the stele of the axis, bending out towards the flower and leaving a gap, shown in figure 7. The flowers are so closely arranged on the spike that there are frequent gaps, so that in any cross section the stele is interrupted by these

in a number of places. This condition is shown in figure 1. Starting with figure 2, the beginning of one flower is shown, the one cut out by the straight lines in figure 1. The lowest surface of the bract is shown in figure 4, as the section of the flower in this case is somewhat oblique. Of course this was unavoidable, as the flowers of *P. lanceolata* are attached to the axis at an angle of about sixty degrees. Figure 6 is higher up on the bract so that the tissue is continuous, and in this figure some of the vessels of the midrib of the bract are shown in longitudinal section. This vascular bundle of the bract gives off two branches, one on either side, forming three veins, suggestive of the large ribs of the foliage leaves of the genus. This series ends with figure 6, since it is too oblique for the study of the flower.

*Vascular Supply.* The inflorescence is a spike, with the flowers spirally arranged on the axis. Each flower is subtended by a bract, which might be either a modified leaf or a part of the flower. In the spikes of some other plants the bracts are really modified leaves, in which case the flowers are axial instead of being part of an inflorescence, as they appear to be. The study of all species of *Plantago* examined shows clearly that the bract is really a part of the floral branch. The vascular supply to the flower separates itself from the stele of the axis, bending outward towards the flower, and from this large vascular bundle a smaller bundle is separated which enters the bract. This bract bundle leaves the main flower bundle after it enters the pedicel, so that there is no doubt about its being an offshoot from the pedicel (fig. 7).

In the development of the flower of *P. lanceolata*, the large vascular bundle enters the pedicel and very soon gives off a bundle to the bract. The stele of the flower at this point appears to be almost a solid mass of xylem surrounded by phloem (figs. 8, 9). At the base of the receptacle the bundles to the sepals are separated in rapid succession, as shown in figures 10-12. Just a little higher up the bundles for the four petals pass out, and almost immediately the four stamen bundles are separated from the stele, directly opposite the sepal bundles. This stage is shown in figure 13. The corolla is soon cut off from the receptacle, but the stamen bundles are still within the receptacle (fig. 14).

After this the stele becomes rather indefinite, and it becomes difficult to distinguish the xylem elements because of the lack of lignification. However, by careful examination with the high-power objective they can be made out, and appear as more or less definitely defined groups. Figure 15 (pistil) shows this condition, in which are seen two dorsal bundles (*db*) and four ventral bundles (*vb*) which supply the carpels.

Following this stage we find the stamens separating from the receptacle as a ring of delicate tissue, and they remain attached as far up as the appearance of the lower part of the third ovule from the base of the placenta. They are never attached to the corolla. In the ovary the dorsal bundles remain distinct and may be traced some distance up through the pericarp,

but the four ventral bundles soon lose their identity and fuse together as one large central bundle in the placenta (figs. 16-21). Each ovule is supplied by a bundle given off from the placental bundle (fig. 26), which passes out at right angles to the main bundle, then passes over and down the outer edge through the raphe.

What remains of the placental bundle passes up into the style and separates into two bundles, which probably represent in each case the two original ventral bundles of a carpel (fig. 29).

### *Plantago major*

There is practically no difference in the history of the vascular supply to organs of this flower as compared with that of *P. lanceolata*. There is a dense substance present in the center of the ovary which practically obscured the vascular tissues after the stamens were cut off. But up to that point it is quite similar to *P. lanceolata*.

The tissues of the sepals show a rather interesting condition. The epidermis has remarkably thick walls, and there is a region at the center on the inner face made up of a broad band of very thick-walled cells which are evidently cutinized. This band is just above the midvein and varies in thickness from three to six cells. The epidermis of the upper or inner face of the sepal is much thicker than that of the outer surface. Another interesting feature is that the mesophyll tissue is made up of large, thin-walled cells with many large air spaces between them, rather suggestive of hydrophytic conditions than of the dry, dusty ones in which this plant is commonly found.

### *Plantago Rugelii*

The most important difference here is in the origin of the stamens. Their vascular bundles come out almost simultaneously with those of the petals, as shown in figure 28. The stamens are not separated from the corolla for some distance up from its base.

The sections of the flower of *P. Rugelii* show quite clearly the mode and sequence of development of the sepals, which do not separate from each other for some distance (about one-fifth of their length) from the base. This condition is shown in figures 30-33. This imbricate arrangement is characteristic of the genus.

### DISCUSSION

An examination of the floral anatomy of the several species of *Plantago* studied seems to show a closer relationship of the Plantaginaceae to the Polemoniales than to the Rubiales. In *Plantago* we find the two-celled ovary with axial placentation which is characteristic of several of the higher families of the Polemoniales, such as the Acanthaceae and Scrophulariaceae, as also the four stamens. However, there is no evidence in the histological study of a reduction from five to four stamens, although the characteristic plan of four probably is a reduction from the plan of five of most of the Polemoniales.

Henslow (6) regards the origin of the vascular supply of the floral organs as axial; he says that the bundles are given off from the axis to the sepals and petals, and that from these are given off the bundles of the inner whorls, stamens and pistils, separated by division either radial or tangential, and that those of the inner whorls are generally within the tissue of the receptacular tube. He says further that they may be given off in one of three ways:

(1) They may be given off at the terminal points of the pedicel, that is at the base of the flower; (2) They may all run parallel from the base to the summit of the receptacular tube; or (3) They may branch off at various heights within the tube itself.

The study of the origin of these structures in the three species of *Plantago* here considered shows that (a) the vascular supply to the floral bract is given off from the floral axis (fig. 4-8); (b) the vascular supply to the sepals is given off at a slightly higher level (figs. 7, 10-13); (c) the vascular supply to the stamens then appears directly opposite the stamens, and at the same time the petal bundles are separated, alternating with the stamen bundles. Each whorl of organs forms a ring of tissue, so that there are three well defined cylinders of tissue in the lower region of the receptacle before the stamens and sepals finally separate into the distinct parts characteristic of the mature flower. The fact that the bract is supplied directly from the floral axis seems rather significant. At a very early stage (fig. 4) the floral axis shows five nearly equal groups of xylem, the outer one passing out to supply the bract, the other four soon losing their identity in the axis. If it is true that foliage leaves were originally sterilized sporophylls (Coulter, Barnes, and Cowles, 4), and if, as Henslow (6) suggests, stamens were split off from sepals, might it be possible that in the floral bract of *Plantago* we have the lost fifth sepal?

As to the primitiveness of the Plantaginaceae, there is a suggestion of aquatic ancestry in the loose, open tissues of the sepals with their thick-walled upper epidermis and thinner-walled lower epidermis. There is, of course, the other genus *Littorella* which is aquatic, and, of our species of *Plantago*, one, *P. decipiens*, is semi-aquatic, so it may be that the family has developed from an aquatic to a terrestrial habit. The plan of four, on which the flower is formed, is rather a specialized condition, and there is a reduction from numerous ovules (as many as 18 in some species) to one in each cell, as seen in *P. lanceolata* and some other species. This suggests another point of interest—the fact, previously mentioned, that the petals are united in a ring from their earliest appearance (fig. 14), which, according to Wernham (8), is an exception to the usual condition in Sympetaleae. Wernham says of this group that “the corolla makes its appearance in the form of separate papillae which fuse at a very early stage.” Such a condition would of course point to a polypetalous ancestry. He adds, however, that “it is by no means impossible for a sympetalous group to have been derived directly from, say, a protangiospermous ancestry without the

ancestry of a polypetalous stock. A tubular perianth whorl need not of *prima facie* necessity be the product of fusion in the course of descent." He states later, however, that he considers it "scarcely probable in case of any known Sympetalae." In view of the above statements, it seems rather difficult to know what interpretation should be placed upon the unusual mode of origin of the corolla of *Plantago*. At least, it presents an interesting question: Is it possible that this is a connecting link between Plantaginales and some remote protangiospermous group, in spite of the evidences of advance shown by other characters?

The spiral arrangement on the spike and the opening of the flowers in spiral order, as well as the spiral order in appearance of the sepals, show a decidedly primitive character.

In *Littorella* the carpels are reduced to one, with a single ovule. There is evidence of advance shown in the cohesion of the filaments of the stamens at their base in *P. lanceolata*, and still further advance by the adnation of the stamens with the corolla in *P. Rugelii*. So, too, the sympetalous corolla and partly coherent sepals are evidence of advance. The membranous nature of the floral leaves is a highly specialized condition.

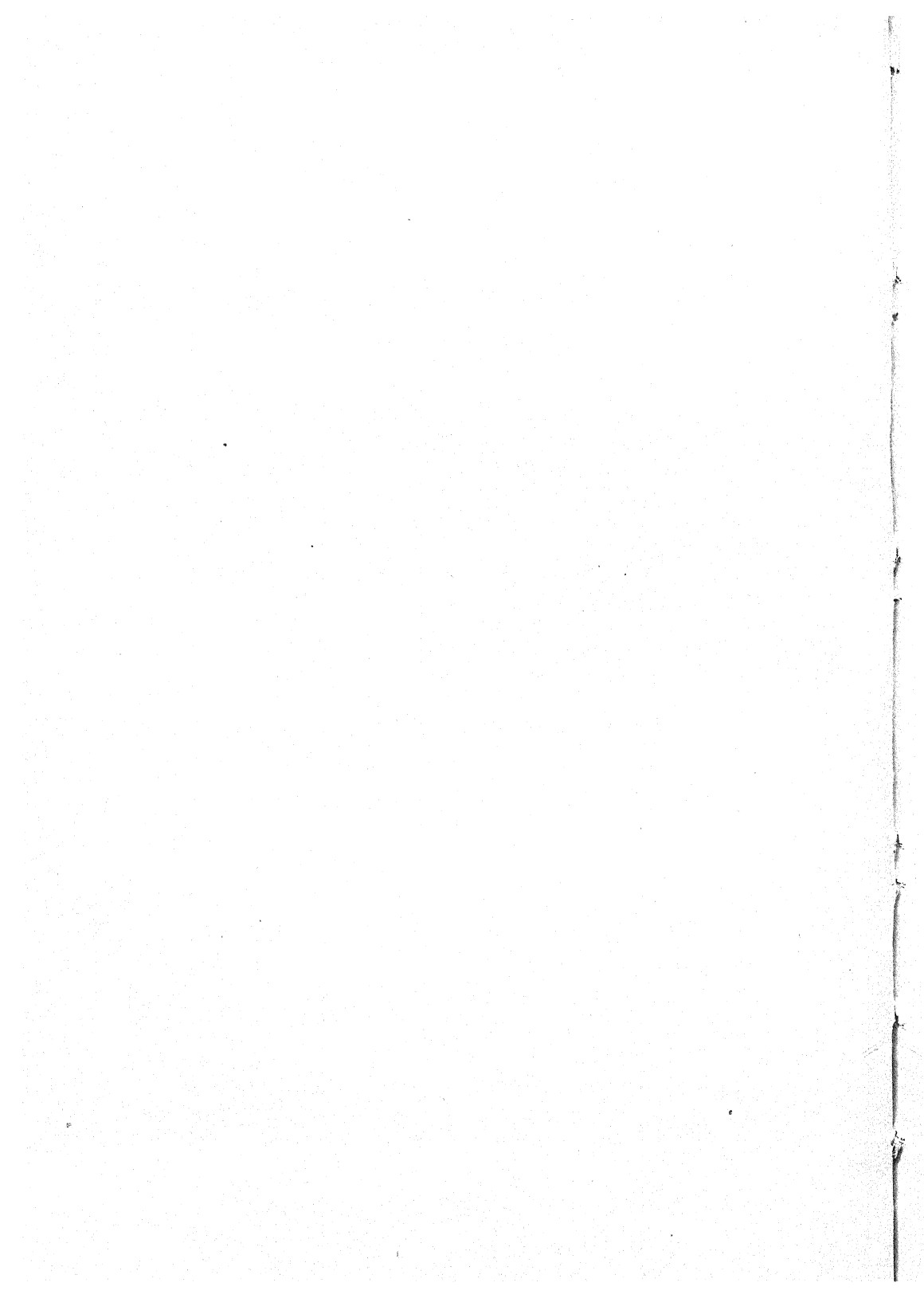
#### SUMMARY

The vascular supply to the various floral organs, including the bract, is from the floral axis, that of the bract being given off earliest, followed almost immediately by the bundles to the sepals, which leave the axis, one at a time, in rapid succession; later follows the vascular supply of the stamens, opposite the petals, and alternating with the stamen bundles the petal bundles; these two whorls have their bundles cut out almost simultaneously.

The characters showing primitiveness are: (1) the nature of the tissue of the sepals, suggestive of water ancestry; (2) the spiral tendency in arrangement of flowers on the spike and the order of appearance of vascular supply to sepals; (3) the possible primitiveness in the origin of the corolla; (4) wind-pollination in most species. Those characters which show advance are: (1) reduction in the number of ovules from eighteen in some species to one in others; (2) sympetalous condition of the corolla; (3) cohesion of sepals and of stamens in some, and (4) adnation of stamens and corolla in others; (5) the membranous nature of the floral leaves. It is quite possible that the aquatic tendency shown by some of the species may be a reversion, that the group may have arisen from an aquatic ancestry and that a few have returned to the aquatic habit, since the great majority of species are terrestrial. On the whole the group seems to show a high stage of advance, though a number of primitive characters still persist.

These studies were carried on in the botanical laboratory of Cornell University, under the supervision of Dr. A. J. Eames, to whom the writer is grateful for his interest and helpfulness.

HENDERSON: FLORAL ANATOMY OF *PLANTAGO*



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## EXPLANATION OF PLATE XXXV

These figures are free-hand drawings, but are accurate as far as relationships of vascular bundles and appearance and separation of the organs in the flower are concerned.

FIG. 1. Cross section of axis of spike, *P. lanceolata*.

FIGS. 2-5. Beginning of flower, four stages; the beginning of the bract shown in figure 5.

FIG. 6. Stage showing bundle traces forming three ribs.

FIG. 7. Longitudinal section through flower of *P. major*, showing vascular supply of flower bract.

FIG. 8. Only lateral traces to bract, central one having bent downward as shown in figure 7.

FIG. 9. Only outer portion of bract, with receptacle in center.

FIGS. 10-12. Receptacle showing, respectively, two, three, and four traces.

FIG. 13. Receptacle showing all sepal traces, petal bundles (*pb*), and stamen bundles (*st*).

FIG. 14. Diagrammatic drawing showing sepals (*sep*) still united but cut off, also petals (*pet*) cut off, and beginnings of stamens (*st*) being cut out of receptacle (*rc*).

FIG. 15. Stage in which carpel bundles first appear, showing two dorsal bundles (*db*) and four ventral bundles (*vb*), stamens (*st*), and stamen bundles (*sb*).

FIG. 16. Fusion of ventral bundles to form one central bundle (*cb*).

FIGS. 17, 18. Stages higher up, showing stamens coherent but cut off from the receptacle, which higher up becomes the ovary.

FIG. 19. Stamens still coherent, ovary forming the lowest ovule (*ov*); ovary bundle (*ob*) and dorsal bundles present.

FIG. 20. Ovary showing one ovule; ovary bundle and dorsal bundles still present.

FIG. 21. Same, showing cutting off of placenta.

FIGS. 22-24. Ovary showing placenta and lower ovule, and later stages.

FIG. 25. Beginning of second ovule.

FIG. 26. Much higher up, showing placenta with its bundle (*fvb*) divided into two regions one of which is giving off trace to ovule.

FIG. 27. Stage higher up.

FIG. 28. Stage in development of *P. Rugelii*, showing sepal traces (*st*), petal bundles (*pb*), and stamen bundles (*sb*), the two latter appearing simultaneously. The vascular stele remains in a broken circle at the center.

FIG. 29. Style, showing two fibro-vascular bundles.

FIGS. 30-33. Corolla with stamens included being cut off from the receptacle; first sepal separating at *s*<sup>1</sup>, also two dorsal bundles (*db*) and four ventral bundles (*vb*) in the receptacle; other sepals indicated by *s*<sup>2</sup>, *s*<sup>3</sup>, and *s*<sup>4</sup>. The last figure shows two ovules partly formed.



# SOME PHYSIOLOGICAL CONSIDERATIONS OF THE "DELICIOUS" APPLE WITH SPECIAL REFERENCE TO THE PROBLEM OF ALTERNATE BEARING

EMERY R. RANKER

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## PURPOSE OF THE INVESTIGATION

One of the most disagreeable and at the same time the most unprofitable features of commercial apple production is the tendency of most apple varieties to "take a rest" every other year. This process is referred to as "off-year" or alternate bearing. Various causal relationships have been suggested from time to time; the conception now most generally held is the idea of a nutritional balance expressed as a "carbohydrate-to-nitrogen ratio."

The various practices in orchard management, such as cultivation, pruning, fertilization, etc., disturb the normal equilibrium of the trees, and readjustments ensue. The factors involved are many, and the ultimate solution of the problem is definitely to determine and to understand them. After this is done it will be possible to regulate these factors to orchard practices in such a way as to cause this "shift of equilibrium" within the tree to go in the direction of higher orchard yields of better-grade fruit at a lowered cost of production. This, obviously, can not be accomplished until the problem of alternate bearing is solved. Alternate bearing in apples is not uniform the country over; it varies as between varieties and locations, being more common in fruit sections in the north and east than in the south or west.

The investigation here reported was carried out under Utah conditions. The problem involved may be more definitely stated in the form of certain questions: (1) To what extent does the "Delicious" tend toward alternate bearing in this section? (2) When does fruit-bud differentiation begin, and when is it completed? (3) What is the performance record of the individual spurs as regards fruit-bud differentiation, fruit set, fruit matured? (4) What physiological effects are produced by thinning of blossoms, thinning of spurs, pruning by heading back, pruning by thinning out, and ringing? (5) What are the fundamental natures of these treatments? (6) To what degree is the Delicious self-sterile? (7) Is the wind effective as a pollinating agent?

## DEFINITION OF TERMS

"Spur" or "fruit spur" will refer to those growths, terminal or lateral, on the twigs or branches that have not exceeded a growth of more than 50 mm. in any one year. This, of course, is a purely arbitrary standard.

"Terminal growth" will refer to the annual growth made only by the individual twigs; it will not include the annual growth made by the spurs.

"Second growth" will have reference to that double condition of annual growth made in two separate parts; the first part made in the spring and terminated by a bud, which did not remain dormant normally, but burst into active growth during the summer to form the second part of the annual growth; this latter being terminated by a bud that remained dormant until the following spring.

"Pruning by heading back" will refer to the removal of the terminal portion of the twig or branch without reference to any particular point of branching.

"Pruning by thinning out" will refer to the removal of a twig or branch at the point at which it branches from a larger twig or branch.

"Ringing" will refer to the removal of a narrow ( $\frac{1}{8}$ – $\frac{1}{4}$  in.) annular ring of bark, as deep as the cambium layer, from the basal portion of the twig, branch, or limb being treated.

## MATERIALS

The materials for this study consisted of 16 Delicious apple trees, located in 3 different orchards. The trees in each of the orchards are of about the same age—approximately 12 years. They are all located under the irrigation canals of this region and receive applications of water at regular intervals. In 1923 the application of water was rather excessive. The orchards have in common also the advantages of excellent air drainage. As a whole the section is very favorable for apple production, although there are years when the apple crop is rather severely damaged by winter injury or late spring frosts. The respective orchards differ in certain respects, and these should be noted:

1. Orchard no. 1. The soil is a rather heavy clay loam high in lime and fertility. For the last 5 or 6 years the orchard has been intercropped with alfalfa, all the crops of which have been removed from the land, the fertility being maintained by annual applications of manure. The general condition of the orchard is very good as regards pruning (mainly thinning out) and general orchard management; the spraying has not been sufficient, however, to overcome the depredations of the fruit-tree leaf-roller. Three trees in this orchard were used for the purposes of this investigation.

2. Orchard no. 2. The soil is a gravelly loam having abundant fertility. The soil management consists of intercropping with strawberries, and the fertility of the soil is maintained by manurial applications. The general condition of the trees is very good. Four trees in this orchard were chosen for pruning studies in connection with limb, branch, and spur treatments.

3. Orchard no. 3. The soil is a fine, gravelly loam under a system of rather continuous covercropping with a mixture of alfalfa and orchard grass and occasionally a year of clean cultivation. The general condition of the orchard is good; the general system of pruning has been mainly that of heading back. In this orchard 9 trees were chosen. The trees are not especially vigorous, vegetatively.

### METHODS

For the purpose of this investigation the twig has been considered as the basis of comparison and the validity of so doing will be considered later in the discussion of results. In order to obtain the desired data certain treatments were given, as follows:

#### Tree treatments:

1. Pruning by heading back.
2. Pruning by thinning out.
3. Pruning by heading back and thinning out.
4. Check on previously mentioned treatments—*i.e.*, no treatment.

#### Limb and branch treatments:

1. Ringing: early ringing, and a later ringing on some branches.
2. Heading back of all branches not previously so treated.
3. Thinning out of all branches not previously so treated.
4. No treatment.

#### Spur treatments:

- |  |  |
|--|--|
| <ol style="list-style-type: none"><li>1. Thinned 50 percent.</li><li>2. Thinned 25 percent.</li><li>3. Thinned 12½ percent.</li><li>4. None removed.</li></ol> | } These treatments were given to various twigs on all trees. |
|--|--|

#### Blossom treatments (given to various twigs on all trees):

1. All removed from each flower cluster *except*:
  - (a) One left, if that many.
  - (b) Two left, if that many.
  - (c) Three left, if that many.
  - (d) Four left, if that many.
  - (e) All left and none removed as a check.

### RESULTS

The data presented in this report are presented in terms of percentages and are based upon the individual performance records of over 20,000 spur and terminal growths, covering the 3-year period, 1922-1924.

#### Degree of Alternate Bearing

Inasmuch as one of the most direct applications of such a study as this is to the problem of alternate bearing, it is well to consider first the extent to which the Delicious has developed the "habit" of alternate bearing (table 1).

TABLE 1. *Degree of "Off-bearing"*

	Orchard			
	No. 1	No. 2	No. 3	Average % <sup>‡</sup>
% of fruit buds opened in 1923 *.....	70.8	42.4	52.4	54.0
% of fruit buds opened in 1924 †.....	0.1	26.0	3.8	8.0

\* Percentages are based on a total of 6,010 spurs and longer growths.

† Percentages are based on a total of 9,847 spurs and longer growths.

‡ Similar results were obtained for the years 1921-1922; 1922-1923.

The figures given in table 1 indicate a rather pronounced tendency toward alternate bearing, and especially so when one considers the fact that the production for the year 1923 was not exceptionally large. These data also show that as the production for 1923 increased (42.4 percent to 70.8 percent) the degree of off-bearing increased, being practically 100 percent in orchard no. 1. Such a high percentage in orchard no. 1 is probably due in part to the serious leaf-roller infestation in that orchard. The data of table 1 and those of table 2 indicate that there are a large number of "boarder spurs" which are forming leaf buds year after year and which very seldom become productive. The practical problem is to put these "drones" to work and profitable productivity; this, however, is another problem than that here considered.

TABLE 2. *Extent of Consecutive Fruiting on the Same Spur \**

	Orchard			
	No. 1	No. 2	No. 3	Average %
% of spurs <i>opening</i> fruit buds.....	2.5	2.5	0.8	1.5
% of spurs <i>not opening</i> fruit buds.....	97.5	97.5	99.2	98.5

\* Fruiting for any two consecutive years is considered "consecutive fruiting." The percentages given are based on a total of 20,318 spurs, the period of observation being 1920-24.

It has been suggested by some investigators that one way to overcome the "off-year" bearing "habit" is to develop the terminal-shoot-bearing tendency. As far as the results of this investigation show, this tendency in the Delicious variety is practically a constant factor regardless of the treatments given and is, for practical purposes, a negligible quantity entirely as shown by the figures of table 3.

TABLE 3. *Extent of Terminal-shoot Fruiting*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% of terminal growths forming fruit buds *.....	8.4	6.6	4.8	5.9

\* The percentages given are based on a total of 1,552 terminal growths.

### Self-Sterility in the Delicious

The fruit-bud clusters used to obtain these data were bagged just prior to the opening of the blossoms. The blossoms opened under the bags in each of the first 2 cases, and when the pistils were judged to be receptive the following methods were used:

1. The bagged cluster, with the bag still attached and intact, was shaken vigorously so as to cause some of the pollen from the anthers of that same cluster to fall on to the pistils.

2. Some of the bags were removed, and the clusters were pollinated by hand with some of the pollen (from flowers bagged previous to opening) (a) from the same bud cluster, (b) from flowers on the same tree, (c) from flowers on a different tree of the same variety (Delicious), and (d) from flowers on a Jonathan tree. This latter pollination was further extended by the fact that the only trees in the orchard, except the Delicious, were Jonathan or Black Ben Davis—the Jonathan being the only variety in the immediate vicinity.

3. Some of the blossoms were emasculated after the method described by Sax (19) and left to be pollinated by natural agencies.

The results obtained from the above-described methods may be summarized by a brief statement: In all cases none of the blossoms set fruit except those pollinated by hand using the pollen from a Jonathan tree, and of those pollinations about 95 percent set fruit, of which 60 percent matured the fruit. This work was carried out in all of the three orchards. Sax (19) suggests the Duchess variety as a good pollinizer for the Delicious. The Jonathan has been found to be a very potent pollinizer for the Delicious under Utah conditions. The Duchess is not grown to any extent in the state.

The self-sterility of apple varieties is very general and well recognized. The work of Lewis and Vincent (14) is typical: Of 87 varieties, 59 were self-sterile, 15 were self-fertile, and 13 were partially self-sterile. It is also known that better crops are produced under systems affording opportunity for cross-pollination, that wind is ineffective in apple pollination, and that bees are essential in transmitting pollen. Ewert (6) concluded from his work that under German conditions, where villages are close together and many kinds of fruits are grown, mixing of varieties in plantings is not necessary. Whitten and Wiggans (21) report that varieties "such as Delicious, Ingram, Ben Davis, Gano, and York appear to be capable of fertilizing their own flowers when planted in large blocks." From present data this does not seem to hold true under Utah conditions as far as the Delicious is concerned.

### Pollination of the Delicious

Tree A-7 in orchard no. 2 is unprotected against the wind from the east, which is the direction of the regular canyon breezes. The nearest trees that could offer any protection from the wind are located 500 feet to the east. Although there was profuse blooming, not a single fruit was set on

the east half (windward side) of the tree. On the protected side of the tree there was an average set of fruit and an average maturity of the fruit. The conditions on the east side were so unfavorable for the insects (principally bees) that they evidently did not succeed in pollinating the blossoms there, and the wind was not effective. Bees and other insects seek the sunshine, but when there is a wind, such as prevailed during the blossoming period of this tree, the insects do not operate and in preference even seek the shady north side for their nectar-hunting grounds. Had the wind been an effective agent in pollination, this tree would have been pollinated to the maximum, for there was sufficient canyon breeze during that period to pollinate hundreds of acres of such trees. The indication is that wind-breaks would be of value to the fruit-grower in this section.

### Fruit-bud Formation and Differentiation in the Delicious

The methods used to obtain the desired data have been those of histological technic adapted largely after the methods of Chamberlain (3). The results are recorded in table 4.

TABLE 4. *Fruit-bud Formation and Condition of Progress*

Date	Condition of Progress of Differentiation
6/15/23.....	All buds leaf buds; no evidence of differentiation.
6/19/23.....	Differentiation just starting in a very few.
7/ 2/23.....	Differentiation rather well started.
7/ 9/23.....	Differentiation well started.
7/24/23.....	Differentiation very well started.
8/17/23.....	Differentiation largely completed and maturation well started in some.
3/15/23.....	Mature buds; both fruit buds and leaf buds fully developed.

In all the buds examined on the various dates, especially those gathered in the later periods, there was no evidence of a second period of differentiation except as noted in the case of second growths (table 14). Goff (8) noted a second period of differentiation (August 1—September 3) in some of the varieties with which he was working. This condition is probably a varietal one, and possibly climatic factors are of influence also. The progress of fruit-bud differentiation in orchard no. 3 was about a week behind that in the other 2 orchards; this difference was due largely to the heavier soil type. Microphotographs were taken to illustrate the above-noted progress of differentiation, but they are not included in this paper, being reserved for a later publication.

The nutritional relationships involved in fruit-bud formation are not definite or so readily determined as are the anatomical changes. Goff (8) states that "in a measure they (leaf buds and fruit buds) are interchangeable." If this be true, it must occur within extremely narrow limits; it is conceivable, however, that differentiation might be halted or completely stopped under certain conditions. Various investigators have indicated and more or less emphasized those factors which they have considered to

be the principal or controlling factors involved in fruit-bud formation. These may be very briefly summarized as follows:

1. Climatic conditions (Goff, 8; Brown, 1).
2. Adequate supply of stored carbohydrates (Kitchen, 12).
3. A highly concentrated sap and wood growth (Whitten and Wiggans, 21).
4. Adequate supply of carbohydrates and nitrogen in a balanced ratio (Kraus and Kraybill, 13; Hooker, 10; Roberts, 17; Gardner, Bradford, and Hooker, 7).
5. Soil moisture (Pickett, 16).
6. Cultural treatments as opposed to fertilizers (Gourley, 9; Drinkard, 5).

It is not meant to imply by this summary that these writers do not recognize the influence of other factors and make allowance for their influence. After a consideration of the entire problem of fruit-bud formation, Gardner, Bradford, and Hooker (7) summarize their remarks as follows:

Regularity of bearing, therefore, is a cultural problem to be dealt with by influencing nutritive conditions. . . . Alternate bearing is not a necessary product of any type of bearing. If nutritive conditions within the tree are favorable fruit buds may be formed every year. Consequently alternate bearing is a problem of nutrition. Different bearing habits are probably associated with different methods or places of food storage.

This opinion is not entirely accepted by all workers. Brown (1) suggests that alternate bearing is not wholly a question of nutrition and cites a case in which "habit" seems to be a potent factor. Butler (2) concludes that pruning is the only really effective means of accomplishing the result of overcoming the problem of alternate bearing. Gourley (9) in 1915 summarized the factors "which are said to cause the production of fruit buds" as follows:

#### *General Causes*

Climatic factors	Accidents or disease
Varietal forms and adaptations	Health and vitality
Age and maturity of the plant	Spraying

#### *Specific Factors*

Large storage tissues	Amount of leaf area the previous season
Grafting on slow-growing stock	Abundant reserve food materials
Drought	Pruning or training the tree
Intense light conditions	Summer pruning
Root-pruning	Thinning the fruit
Ringing or girdling the branch or trunk	Reducing the amount of nitrogen
Bending, twisting, or breaking the branch	Basic slag fertilizer
Defoliation	Phosphorus and potash fertilizers.

### **Results of the Various Treatments Given**

In the interpretation of the data in the following tables one condition must be noted, namely, that all treatments were given during the period May 20-25, 1923; therefore any data given under the heading "blossoms formed" for the year 1922 are the results of no special treatment, since these blossoms were formed during the early summer of 1922 before any of these treatments had been given. The data given under all the other

headings, however, are valid as being recorded under the particular treatment being considered.

The data of tables 5-15 are given primarily for a two-fold purpose: (1) To indicate something of the fundamental nature of the treatments given; and (2) to demonstrate a practical method of determining whether or not, as a result of the treatments given, the so-called "shift of equilibrium" within the trees is tending toward an increased and more uniform productivity. The results are expressed in terms of percentages.

### Responses to Ringing

TABLE 5. *Productivity Responses of Spurs Borne on Twigs which were Ringed*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1922 *.....	58.3	48.7	53.7	53.4
% fruit set 1923 *.....	41.3	46.1	37.5	41.2
% fruit matured 1923 *.....	29.8	36.9	33.5	33.5
% blossoms formed 1923 †.....	0.7	63.5	11.0	20.7

\* Percentages given are based on a total of 889 spurs.

† Percentages given are based on a total of 2,513 spurs.

When table 5 is compared with table 9, it is evident that the rate of fruit set and fruit matured for the treatment of ringing is very high. The relative percentage of fruit set was highest in orchard no. 2 (practically 100 percent of the blossoms formed) and lowest in orchard no. 3 (not quite 70 percent of the blossoms formed). The relative percentage of fruit matured (using the percentage of "fruit set" as a basis of calculation) was highest in orchard no. 3 (practically 90 percent) and lowest in orchard no. 1 (slightly over 70 percent), which fact means that the "June drop" was light in the former and heavier in the latter.

TABLE 6. *The Effect of an Early Ringing as Opposed to a Later Ringing*

	Ringed 5/25/23 *	Ringed 7/4/23 †
% fruit buds formed on ringed twigs.....	4.0	14.4

\* Percentages given are based on a total of 137 spurs.

† Percentages given are based on a total of 157 spurs.

The data of table 6 are directly comparable, since the ringing was done on adjacent twigs and branches of the same trees located in the same orchard. As a result of the treatment of ringing, certain fruit characteristics must be noted also: (1) Fruit from orchard no. 2 was markedly increased in size and quality. (2) In orchard no. 3, early ringing (May 25) produced very small, hard, green-colored, cull fruit; if the treatment was given later (July 4), large, fine-colored fruit was produced, being as much larger than the



check fruit as the early-ringed fruit was smaller than the check fruit. (3) Fruit produced below the ring (*i.e.*, on the trunk side) was much larger and of a better quality than the fruit produced above the ring or on untreated twigs; this condition maintained in orchard no. 3 regardless of when the treatment was given. These facts are important as being indicative of the fundamental nature of the treatment, and of the nutritional condition of the orchards. They will be referred to later.

### Responses to Pruning by Heading Back

TABLE 7. *Productivity Responses of Spurs Borne on Twigs Pruned by Heading back*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1922 *	68.8	40.9	42.0	48.3
% fruit set 1923 *	36.4	33.2	15.8	25.8
% fruit matured 1923 *	22.1	16.1	11.9	15.6
% blossoms formed 1923 †	0.0	9.3	0.0	1.9

\* Percentages given are based on a total of 1,020 spurs.

† Percentages given are based on a total of 2,162 spurs.

By comparison of the data of tables 5 and 7, the indications are that the treatments of ringing and pruning by heading back are directly opposed as regards the effects produced upon the nutritive balance within the tree; this is shown especially by the data for "blossoms formed 1923." In all cases (notes for table 14) pruning by heading back produced a greatly increased vegetative response. The apples which persisted and grew to maturity, however, were uniformly larger and of a better quality than the check fruit.

### Responses to Pruning by Thinning out

TABLE 8. *Productivity Responses of Spurs Borne on Twigs Pruned by Thinning Out*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1923 *	—	6.9	—	6.9

\* Percentages given are based on a total of 303 spurs.

The apples produced on trees pruned by thinning out were not significantly different from the check fruit as regards size and quality. This condition would indicate that this treatment disturbed the nutritional balance within the tree least of all the treatments given.

## Responses to No Treatment (Check)

TABLE 9. *Productivity Responses of Spurs Borne on Twigs Given no Treatment*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1922 *.....	80.7	—	45.7	64.6
% fruit set 1923 *.....	32.5	—	13.7	23.8
% fruit matured 1923 *.....	16.5	—	9.8	13.4
% blossoms formed 1923 †.....	0.0	24.3	0.7	4.4

\* Percentages given are based on a total of 731 spurs.

† Percentages given are based on a total of 2,299 spurs.

In connection with table 9 it is of interest to notice that only in orchard no. 2 were fruit buds formed to any extent in 1923; the trees of this orchard are moderately vigorous, vegetatively. In orchard no. 1 the trees are excessively vigorous, vegetatively, and below normal in vigor in orchard no. 3. From the data thus far presented, these orchards may be divided into 3 classes (see "Discussion," p. 419).

## Responses to Removal of Spurs

TABLE 10. *Productivity Responses of Spurs, Remaining after 50% of the Total Number had been Removed*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1922 *.....	69.7	41.6	47.8	53.5
% fruit set 1923 *.....	36.5	35.6	21.9	29.7
% fruit matured 1923 *.....	27.4	19.9	18.0	21.5
% blossoms formed 1923 †.....	0.3	24.1	3.2	8.1

\* Percentages given are based on a total of 740 spurs.

† Percentages given are based on a total of 2,191 spurs.

TABLE 11. *Productivity Responses of Spurs, Remaining after 25% of the Total Number had been Removed*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1922 *.....	70.6	45.6	47.5	54.9
% fruit set 1923 *.....	39.6	40.4	21.5	31.5
% fruit matured 1923 *.....	23.7	20.7	18.8	20.8
% blossoms formed 1923 †.....	0.4	31.3	4.4	9.4

\* Percentages given are based on a total of 941 spurs.

† Percentages given are based on a total of 2,488 spurs.

TABLE 12. *Productivity Responses of Spurs, Remaining after 12½% of the Total Number had been Removed*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% blossoms formed 1922 *.....	72.9	46.5	45.2	55.1
% fruit set 1923 *.....	32.2	41.9	22.6	29.9
% fruit matured 1923 *.....	16.1	31.8	17.5	19.8
% blossoms formed 1923 †.....	0.0	35.8	4.7	10.4

\* Percentages given are based on a total of 959 spurs.

† Percentages given are based on a total of 2,598 spurs.

In tables 10-12 the rather marked uniformity of results obtained is noteworthy, regardless of the severity of the treatment given. This is illustrated especially by the fact that, on comparing the data of the "average %" column, the greatest differences found between any two comparable percentages are 1.8 in the case of "fruit set 1923," 1.7 in the case of "fruit matured 1923," and 2.3 in the case of "blossoms formed 1923." This same uniformity was noticeable also as regards the size and quality of the fruit produced. These data are considered to be quite significant as indicative of the location of stored food and will be discussed later.

TABLE 13. *Recapitulation of the "Average %" Data as Recorded in Tables 5-12*

	Ring- ing	Pruning by		Check	Removal of Spurs		
		Heading back	Thinning out		50%	25%	12½%
% blsms. formed 1922 *.....	53.4	48.3	—	64.6	53.5	54.9	55.1
% frt. set 1923.....	41.2	25.8	—	23.8	29.7	31.5	29.9
Amount of decrease.....	12.2	22.5	—	40.8	23.8	23.4	25.2
% frt. matured 1923.....	33.5	15.6	—	13.4	21.5	20.8	19.8
Amount of decrease.....	7.7	10.2	—	10.4	8.2	10.7	10.1
% blsms. formed 1923.....	20.7	1.9	6.9	4.4	8.1	9.4	10.4

\* The percentages for "blsms. formed 1922" have no significance with respect to any special treatment; attention has been called previously to this situation.

With reference to table 13 it should be noted that the "amount of decrease" of "fruit set" and "fruit matured" is least in the case of "ringing" (refer to tables 5-9). In other words, ringing of the twig or branch produced conditions most nearly optimum for the fertilization of the flowers and the subsequent setting and maturing of the fruit. The "amount of decrease" between the percentages of fruit set and the percentages of fruit matured indicates the number of fruits affected by the "June drop"; this was least again in the case of the ringed treatment. Too much importance must not be attached to the little difference between the "heading back" and the "no treatment," or check, percentages for the year 1922 and for

"fruit set 1923," because of the relatively short time between the date of treatment (May 20-25, 1923) and the setting of fruit. This is partially true of the treatment of ringing and the spur treatments, but not so largely so as in the case of the other two treatments. The general trend of the responses is clearly indicated, however, and the final adjustments are recorded under "% blsms. formed 1923." These data are a measure of the responses which the trees made to the treatments given, in order to establish again a condition of equilibrium and nutritional balance. It seems logical, then, to regard these responses as indicators of the so-called "shift of equilibrium" and as a valuable measure of the relative values of these various treatments (ringing, pruning by heading back, and pruning by thinning out) as opposed to no treatment.

### Responses to Thinning of Blossoms

The blossom treatments consisted of the thinning of the blossoms in each of the flower clusters according to the plan already given in the discussion of methods. The Delicious is self-thinning in this section; no direct responses to these treatments, therefore, were obtained. By the term "self-thinning" reference is made to the ability of the Delicious to drop its blossoms down to a maximum of usually one to the spur. This occurs very early in the spring, soon after the process of fertilization is completed and the young apples have formed (not larger than  $\frac{1}{8}$ - $\frac{1}{4}$  in. in diameter). This is the usual process but infrequently 2 or 3 of the fruits reach the size of  $\frac{3}{4}$ -1 in. in diameter before they drop off; occasionally a few of them in this double or triple condition may mature on the tree.

The first symptom to indicate that a blossom will drop is a pale yellowed discoloration at the base of the young fruit stem. When the stem becomes about  $\frac{2}{3}$  discolored, the least agitation dislodges the newly formed fruit and it drops off. This process is completed within about the period of a week. It is not due to a lack of fertilization, but seems to be due entirely to the fact that in each blossom cluster there is usually only one dominant blossom (centrally located) which opens and is pollinated a day or two before the other blossoms of the same cluster. This blossom grows so much faster than the others that the latter are mechanically broken off by the force of expansion and cell growth of this dominant blossom. Such a situation seems to arise in consequence of the following conditions: (1) All growth in the spring, until the formation of leaves at least, is made at the expense of stored food. (2) The amount of this food is limited. (3) Most of it is utilized by this one dominant, centrally located blossom. (4) The plant (apple tree) adjusts itself to these conditions by diminishing the demand for its stored food by means of the formation of an abscission-layer at the base of those young fruits least able to "demand" their "share." (5) The continued expansion and growth of this dominant blossom mechanically forces its less fortunate sister blossoms to drop off.

The self-thinning ability of the Delicious is very desirable from the standpoint of the grower, and it seems to be a fixed and constant characteristic under Utah conditions. The inflorescence of this variety is dominantly cymose—with practically every exception to this condition it was noticed that 2 or 3 apples were retained, and some of these doublets and triplets grew to maturity. Such apples were inferior as regards size and quality. The period of blooming was May 20-26 (1923).

### Development of Second Growth

In all the orchards there was a period of second growth beginning the first part of July, 1923, and extending irregularly until the first part of August. This condition was of very common (22.4%) occurrence in orchard no. 1 and moderately common (0.7-0.8%) in orchards no. 2 and no. 3, largely because of a period of over-irrigation during the latter part of the summer. The condition in orchard no. 1 was made worse by the leaf-roller infestation which greatly decreased the leaf area of the trees and thus further disturbed the nutritional balance within the trees. These factors, however, are constant for all trees in the same orchard, and the results given in table 14 are comparable as showing the responses to the different treatments.

TABLE 14. *Number of Second Growths from Different Treatments, 1923*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% of second growths:				
On twigs which are <i>ringed</i> *.....	3.3	0.9	1.6	1.7
On twigs pruned by <i>heading back</i> †.....	9.8	8.1	21.7	17.0
On twigs pruned by <i>thinning out</i> ‡.....	—	3.4	—	3.4
On twigs given <i>no treatment</i> (check) §.....	22.4	0.8	0.7	5.2

\* Percentages given are based on a total of 3,907 annual growths.

† Percentages given are based on a total of 3,369 annual growths.

‡ Percentages given are based on a total of 416 annual growths.

§ Percentages given are based on a total of 2,631 annual growths.

The data of table 14 show that in each case, except in orchard no. 1, the treatment of pruning by heading back produced a very high percentage of second growths as compared with the growths of other treatments and with the check trees. The undesirability of this tendency to produce second growths is shown by the data of table 15. In the case of orchard no. 1 (table 14) pruning by heading back very materially decreased the number of second growths as compared with the check trees. This fact indicates that the carbohydrate supply was so low that removal of a portion of each twig (50% heading back in this case) tended to develop more nearly an optimum nutritional balance within the tree. This apparent

anomaly was brought about, no doubt, by the leaf-roller infestation of the trees in this orchard, the leaf area being least toward the terminal end of each twig. It apparently was more economical from the standpoint of nutrition to remove half of the annual twig growths than not to remove them and thus force the tree to develop new leaves on these twigs. Such a condition is abnormal, and in all other cases pruning by heading back produced a greatly increased vegetative growth.

TABLE 15. *Number of Second Growths Forming Fruit Buds, 1923*

	Orchard			
	No. 1	No. 2	No. 3	Average %
% of fruit buds formed:				
On twigs which were <i>ringed</i> * . . . . .	0.0	25.0	2.5	4.6
On twigs pruned by <i>heading back</i> † . . . . .	0.0	0.0	0.0	0.0
On twigs given <i>no treatment</i> (check) ‡ . . . . .	0.0	0.0	0.0	0.0

\* Percentages given are based on a total of 65 second growths.

† Percentages given are based on a total of 574 second growths.

‡ Percentages given are based on a total of 138 second growths.

The data of table 15 indicate that the "no treatment" condition of an orchard allows of no fruit-bud formation on second-growth wood. The same is true of the treatment of heading back. This indicates a decided loss to the grower when such a condition is allowed to develop in his orchard. The treatment of ringing corrected this to a certain extent, but this treatment can not be made a general practice; it is of interest to note also that the corrective concentrating influence of this treatment was not sufficient measurably to overcome the adverse factors stimulating second growths, with the consequent non-formation of fruit buds. The condition of nutritional maladjustment which induced this second-growth response is also somewhat responsible for the large decrease in the number of fruits set for the treatments of "heading back" and "no treatment" as recorded in table 13 for the year 1923.

### DISCUSSION

From a study and comparison of the foregoing data several points stand out rather clearly. The data of tables 1, 2, and 9, in connection with the general vegetative condition of the orchards, allow of the placing of the orchards into three classes:

1. Very vegetative, unfruitful, and with a high degree of off-bearing; orchard no. 1 is representative of this class.
2. Moderately vegetative, fruitful, and with a medium degree of off-bearing; orchard no. 2 is representative of this class.
3. Subnormally vegetative, unfruitful, and with a relatively high degree of off-bearing; orchard no. 3 is representative of this class.

All the data presented are merely the measurable responses that the trees have made to the conditions in the various orchards and to the treatments given. These responses are indicative, therefore, of:

1. The fundamental nature of the treatments given.
2. The influence of those treatments on the "shift of equilibrium" within the tree.
3. The nutritional conditions of the trees.

Before considering the nutritional significance of these responses it is well to indicate again the work of certain investigators who have presented evidence to show the value of a nutritional balance to fruitfulness. Hooker (10) in summarizing some of his work states:

Conditions leading to high starch and low nitrogen content at the time of fruit-bud differentiation were found essential for productivity. Fruit-bearing spurs that developed leaf buds had a low starch and a high nitrogen content, and barren spurs a low starch and a low nitrogen content. The starch-nitrogen ratio is more indicative than the total carbohydrate-nitrogen ratio.

Kraus and Kraybill (13) further emphasize the importance of a nutritional balance in their statement that "fruitfulness is associated neither with highest nitrates nor with highest carbohydrates but with a condition of balance between them." They further point out that there is a direct relationship between the moisture supply and the available nitrogen and that "irrigation or moisture supply is effective in increasing growth or fruitfulness only when accompanied by an available nitrogen supply and *vice versa*."

The work of Hooker and Kraus and Kraybill was based on chemical analyses. This does not mean that the fruit-grower must make chemical determinations of the fruit spurs at regular intervals. On the contrary, when the fundamental nature of the individual treatments is known, when the forces which they put into play within the plant are understood, then the responses which the plant makes to those treatments and combinations of treatments will serve as indicators of the nutritional balance within the tree. This fact would serve as a practical guide for the fruit-grower in his application of cultural practices. Every cultural practice, therefore, if it is to be applied intelligently, must be tested on this basis: How will it affect the nutritional balance within the tree, and to what extent?

No attempt has been made in this investigation to determine chemically this nutritional balance, which is usually expressed as "carbohydrate-to-nitrogen ratio." This idea is rather generally accepted and is valuable as a principle and aid in further nutritional studies and comparisons. In the light of this principle an analysis of the data presented in this report will be attempted.

The data obtained as responses to the treatment of pruning by heading back are quite significant. This treatment produced in all cases (except that of excessive second growths in orchard no. 1 previously referred to in table 14) a greatly increased vegetative response (notes at ends of tables 7

and 14), a high percentage of blossom-abscission and drop (table 7), a relatively heavy "June drop" (table 13), a notable increase in the number of second growths in all orchards except no. 1 (table 14), and a failure to differentiate sufficient fruit buds in 1923 to be of note (table 13). The fruit that persists and matures on a tree pruned by heading back, however, is uniformly larger, though the usual tendency is toward a marked reduction in quantity. These responses show that the treatment of heading back is of such a nature as to cause the tree to use its carbohydrate food for the development of new vegetative growths rather than for the differentiation of fruit buds. In the light of the above-stated principle of nutrition, these responses would indicate that pruning by heading back tends to decrease the carbohydrate-to-nitrogen ratio in such a way as to make for a condition of unfruitfulness. It follows that it would be unwise to apply this treatment in an orchard where the carbohydrate-to-nitrogen ratio is low already, unless it is lower (as in orchard no. 1, table 14) than this treatment would force it.

An analysis of the data on the treatment of ringing shows that this treatment is directly opposite to the treatment of pruning by heading back. Ringing has a concentrating influence; it retards the factors that make for excessive vegetative growth (tables 5, 7, and 13); it greatly decreases the blossom-abscission and "June drop" (table 13), and in every case the formation of fruit buds was increased. According to the nutritional balance within the tree previous to treatment, ringing may either increase or decrease the size of the fruit. These responses would indicate that ringing tends to increase the carbohydrate-to-nitrogen ratio, its influence being opposite to that of heading back. The results obtained will vary also with the time of treatment.

The treatment of pruning by thinning out assumes a position between the two extremes of heading back and ringing, but slightly nearer the former. The vegetative responses are such as to indicate that this treatment tends to disturb the nutritional balance least of any of the treatments thus far considered (tables 5, 7, 8, and 13).

The observations as to irrigation water applied during 1923 indicate that it was somewhat over-abundant. Especially is this true of orchards nos. 1 and 3. Associated with this excess of water in orchard no. 1 was an excessive vegetative growth, but in orchard no. 3 the vegetative growth was not excessive. Also, in orchard no. 3 the condition of the fruit borne on terminal portions (*i.e.*, above the ring) of the twigs that were ringed indicates that the concentrating influence of this treatment produced an adverse response, especially if ringed early. In the light of the findings of Kraus and Kraybill (13) and others regarding the relation between moisture supply and available nitrogen, these responses would indicate that there is a lack of available nitrogen in orchard no. 3 and a need of building up and maintaining the soil fertility.



The foregoing discussion of the various treatments may be summarized as follows:

1. If the carbohydrate-to-nitrogen ratio is above the optimum for fruitfulness, then the application of water, nitrogen fertilizer, or pruning by heading back would tend to improve conditions. If this ratio is below the optimum and lower than any of these 3 treatments would force it (table 14), then the increased application of these treatments would tend to make conditions worse.

2. If this ratio is below the optimum, then any concentrating treatment such as ringing would be beneficial. If this ratio is above the optimum such a treatment would be detrimental.

The vegetative responses that indicate a high carbohydrate-to-nitrogen ratio are: limited vegetative growth, the retention of a high percentage of all pollinated blossoms, a high set of fruit, and a small "June drop" followed by a large crop of small apples borne in doublets and triplets and a rather high degree of alternate bearing.

The vegetative responses that indicate a low carbohydrate-to-nitrogen ratio are: abnormal abscission of blossoms, heavy dropping of newly formed fruit, heavy "June drop" (the apples that do mature are of a large size), and a large percentage of second growths, followed by a practical failure to differentiate fruit buds for a crop the following year.

It seems evident that cultural practices must be based on the nutritional needs of the trees rather than on the usually followed "rule of thumb." The fruit-growers of the past have based their cultural practices on a rather definite and fixed system without much effort to make those practices fit the conditions in their respective orchards. The fruit-grower prunes his trees after a certain fashion and according to the general recommendations of someone he accepts as an authority on the subject, or because his neighbors prune their trees that way. He applies other cultural practices after much the same fashion, that is, he does not experimentally develop the possibilities of better fitting his cultural practices to his own particular conditions. The successful fruit-grower of the future must recognize the needs of his trees and adjust his cultural practices accordingly. He must be somewhat of an experimenter, at least to the extent of finding out exactly the conditions under which he is conducting his business. In this connection it is hoped that the data showing vegetative responses, as presented in this report and in relation to the above-mentioned principle of nutritional balance, will serve as a practical guide to aid the grower in this direction. Illustrations of this point are at hand.

The data of table 14 show that 22.4 percent of second growths was produced in orchard no. 1 with "no treatment"; this was reduced to 9.8 percent by the treatment of pruning by heading back. The only pruning given this orchard for the last 4 or 5 years has been that of thinning out, and the trees are rapidly becoming too tall for economical harvesting of

the fruit. The year this condition maintained (1923), therefore, would have been the opportune time to head back the trees rather severely and at the same time to assist the trees to regain their optimum nutritional balance.

Another example: The results obtained in orchard no. 3—an excessive amount of irrigation water failed to produce more than a moderate vegetative response; the concentrating influence of ringing produced adverse results—indicate the need of building up the fertility of the soil and maintaining it by some system of soil management different from that in use for the last 5 or 6 years (continuous cover crop of orchard grass and alfalfa). Other similar instances are revealed upon a study of the data presented.

Attention should be called to the data in tables 10–13 in connection with the location of stored food in the trees. The data there presented have to do with the responses to the various spur treatments. It will be noticed that the results are practically the same regardless of the treatment given and indicate that the principal place for the storage of the food elaborated by the leaves of any particular spur is in that spur. This condition is also indicated by the uniformity of the fruits produced on the spurs of twigs so treated. If this were not the case, there would have been a far greater difference in the results obtained from such widely varying treatments. It might be suggested that these responses may indicate that none of the food, thus photosynthesized, was stored there. This could not be the case because of the fact that if it were not stored in the spur it would of necessity be stored somewhere else; if stored somewhere else, then the removal of the spurs would allow of a greater volume (twice as much in the case of a removal of 50 percent of the spurs) of food to the remaining spurs, which added supply would of itself produce far more widely varying and consistent results than were obtained. Either this explanation must be correct or no elaborated food was available for storage, and the latter condition is not probable at that stage of the annual development of the tree.

The responses recorded in tables 10–13, as results of the various spur treatments, indicate also the functional individuality of the spurs on the same tree and even on the same twig. This individuality is further shown by the unequal development of spurs on the same twig due to differences in light intensities at the various points. Bradford (11), considering the factors determining fruit-bud formation, states:

It is shown here that the spur or the whole tree or branches may be units and that the individual spur is influenced, sometimes at least, by the performance of the other spurs. How direct this influence is, through effects on near or remote portions of the tree, it is not yet determined. It is shown that the collective performance of the spurs has an influence on rather remote parts of the scaffold limb. Whether the parts have an influence on the spurs is not yet shown definitely.

The work of Chandler (4), Roberts (18), Gardner, Bradford, and Hooker (7), Hooker and Bradford (11), Goff (8), Whitten and Wiggans (21), Kitchen

(12), Magness, Edminster, and Gardner (15), and others further demonstrates this individuality.

One other point should be mentioned at this time. In connection with the formation of fruit buds it is of interest to note that differentiation begins about the time of the occurrence of the longest day of the year (June 21 or 22). At this time active photosynthesis and the accumulation of carbohydrates may continue for the longest period during each day. This is the critical period in the productiveness of the tree. At this time one of two developments may take place, depending upon the nutritional balance within the tree: (1) the rapidly elaborated carbohydrate food may be at once utilized in vegetative responses (manifest by continued growth of vegetative parts or the development of second growths); or (2) this food may accumulate and be utilized in the formation of fruit buds. The two processes do not go on at the same time, as evidenced by the fact that if differentiation of fruit buds occurs it does so at about the time that active wood growth stops.

#### SUMMARY

1. The degree of alternate bearing is quite high in the Delicious variety in this section. Fifty-four percent of the spurs opened fruit buds in 1923, 8 percent opened fruit buds in 1924. The degree of alternate bearing increased as the degree of over-production increased for any one year, and varied considerably as between orchards, being greatly influenced by the nutrition of the tree. This variability suggests the possibility of control.

2. The differentiation of fruit buds on the Delicious apple tree begins about June 19 and continues to about the last of August. No second period of fruit-bud formation was observed except in a few isolated cases of buds on second-growth wood.

3. The period at which differentiation of fruit buds occurs is a critical period in the productiveness of the tree. If it begins, it does so at about the time that active wood growth ceases and at the period of maximum length of day and maximum sunlight. At this period one of two developments may take place: (1) the differentiation of fruit buds and subsequent fruitfulness may result, or (2) continued or renewed vegetative growth may result.

4. Delicious apple trees tend to bear consecutively on the same spur to a very limited extent, only 1.5 percent of the spurs fruiting consecutively for two years or more. There is a high percentage of "boarder spurs" on the Delicious trees in this section that seldom bear fruit and that usually form only leaf buds year after year.

5. The responses of the tree to the various spur treatments were practically the same regardless of the variability of the treatments given. This indicates the functional individuality of the spurs and also the probable location of stored food, *i.e.*, in the spur whose leaves elaborated the food.

6. The tendency toward terminal shoot-bearing in the Delicious is

practically negligible, being 5.9 percent on the average for a period of 5 years. This seems to be a rather constant factor for this variety regardless of the treatments given.

7. The Delicious is definitely a self-thinning variety in this section. The importance of this characteristic and the factors involved are discussed.

8. Wind is ineffective as a pollinating agent. Wind-breaks in many instances would improve conditions for insect-pollination. Insects are the only agents effective in pollination as observed under the conditions of this investigation.

9. The self-sterility of the Delicious is 100 percent under the conditions of this study. The Jonathan is an excellent pollinizer for the Delicious, and the blooming periods of these two varieties coincide, the blooming period of the latter being from 7 to 9 days.

10. The fundamental nature of certain treatments (pruning by heading back, pruning by thinning out, and ringing) is demonstrated and discussed in relation to their effect on the nutritional balance within the tree. The practical value of the vegetative responses which the tree makes to these treatments is discussed, and the application of them is illustrated.

It is with pleasure that the writer expresses his indebtedness to Prof. T. H. Abell and Dr. B. L. Richards, of the Utah Agricultural College, for the appreciated coöperation and suggestions given and the laboratory facilities extended; to Mr. John Lundberg and Mr. Floyd Adams, and Mr. S. W. Yonkers for the use of their orchards; to Mrs. Irene T. Ranker for her untiring assistance; and to Dr. B. M. Duggar, of the Missouri Botanical Garden, for the valued criticisms and suggestions given and for the ever-willing coöperation extended incident to publication.

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# HETEROTHALLISM IN *ASCOBOLUS CARBONARIUS*

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## INTRODUCTION

The problem of sex in the fungi has occupied the attention of mycologists for many years. After Blakeslee's discovery of heterothallism in many of the Mucorineae, workers began with a new zest to determine whether or not the same phenomenon holds for other classes of fungi.

Many interesting results have been obtained in the Basidiomycetes, mainly through the work of Miss Bensaude (1), Kniep (10, 11, 12), Miss Mounce (13, 14.), Buller (2), Hanna (8), Vandendries (16, 17, 18), Miss Newton (15), and others.

Although heterothallism has become an established fact in the Zygomycetes and higher Hymenomycetes, the acceptance of the idea of its occurrence in the Ascomycetes has not been widespread. This doubt is being rapidly removed by several workers. Dodge (6) showed that two separate strains are necessary for the complete formation of sexual fruit bodies in *Ascobolus magnificus*. Edgerton (7) reported the occurrence of plus and minus strains in the genus *Glomerella*. Miss Wineland (20) caused the production of mature perithecia of a species of *Gibberella* when she placed certain strains together. Burger (3) reports a "pseudo-heterothallic" condition in *Cunninghamella*. Kirby (9) reports the occurrence of two strains in *Ophiobolus cariceti*. Wehmeyer (19) gives a historical summary of sexuality in the Ascomycetes.

## MATERIAL, METHODS, AND RESULTS

*Ascobolus carbonarius* Karsten is found growing naturally on burnt-over ground. Soil collected from these areas, placed in pans or glass dishes with moist paper towels underneath, will produce mature apothecia if kept at room temperature. The time of appearance of the apothecia varies from 15 to 21 days.

In Ascobolaceae the spores are ejected from the hymenium for a considerable distance. By placing slides over the apothecia, spores can be obtained in large quantities. These spores can be kept indefinitely in a viable condition.

Various culture media were tried, but a soil decoction made from soil collected from burnt-over ground was the one which proved most satis-

<sup>1</sup> Published, at the expense of the author, out of the order determined by the date of receipt of the manuscript.

factory. A quantity of soil was brought into the laboratory, placed in a dry oven, and sterilized from 8 to 10 hours at 180° C. While this was still warm, hot water was poured over it, the water filtering through until a dark solution was secured. To a liter of this solution was added 25 grams of agar-agar. This was steamed in the autoclave for one half hour, then placed in sterilized test tubes and autoclaved for one half hour at 15 pounds' pressure.

Petri dishes were used for culture work. After the agar decoction was poured into the dishes, spores were sown from the glass slides. The dishes were then placed in a dry oven and gradually heated to from 80° to 95° C. Although the higher temperatures killed many bacteria, lower temperatures around 80° C. proved most satisfactory.

Spores heated to the latter temperature at night showed good germination the next morning. Within two or three days conidia had formed. The function of these and of the sexual organs are fully discussed by Dodge (5).

Although hundreds of cultures were grown to the conidial stage, I have never been able to secure sexual organs, ascogonia and antheridia, in petri dishes. Therefore, all my results in testing heterothallism were secured by first germinating the spores as described above, and then transferring a block of agar containing mycelia to sterilized soil in jelly glasses, moistened with distilled water. On this natural medium the apothecia developed in from 15 to 20 days.

Thirty-seven many-spore cultures were made, the spores being selected at random. After germination they were placed on sterilized soil in jelly glasses. Twenty-one developed apothecia, 16 did not. Whether the failures were due to the strains present being either all minus or all plus was not determined. The 16 failures may have been due to poor conditions, since in some cases molds developed and in others the water supply was too great.

After the results just described were obtained, it was decided to test the fertility of one-spore cultures. Single spores were removed from the slide, either with a capillary pipette or by a needle point made of platinum wire. The latter method was more satisfactory. Each spore was placed in a separate petri dish and germinated as described above. Many of the single spores failed to germinate. This failure may have been due to injury in removing them from the slide to the petri dish. One hundred and fifty one-spore cultures were obtained and placed on soil in jelly glasses. None of these produced apothecia.

The negative results of one-spore cultures led to the testing of strains. Six one-spore cultures were made and labeled as strains 1, 2, 3, 4, 5, and 6. The mycelia were allowed to grow until the agar was covered. Blocks of the agar about one inch square, with the mycelia growing on it, were cut from the dishes and placed in sterilized jelly glasses. All possible combinations of the six spores were used. The combinations totaled 21.

An examination of table 1 will show that the strains when grown by themselves are sterile. Strains 1, 2, 3, and 5 are inter-sterile when placed together, as are strains 4 and 6, but a combination of any one of either group with any one of the other group produces a fertile culture.

TABLE I

Strains Used	No. Cultures	Results *	
		Positive	Negative
1 + 1	5	0	5
1 + 2	15	0	15
1 + 3	15	0	15
1 + 4	8	8	0
1 + 5	20	0	20
1 + 6	24	24	0
2 + 2	5	0	5
2 + 3	20	0	20
2 + 4	21	21	0
2 + 5	20	0	20
2 + 6	5	5	0
3 + 3	28	0	28
3 + 4	10	10	0
3 + 5	16	0	16
3 + 6	5	5	0
4 + 4	15	0	15
4 + 5	8	8	0
4 + 6	10	0	10
5 + 5	5	0	5
5 + 6	20	20	0
6 + 6	11	0	11

\* Results are considered positive when apothecia, and negative when no apothecia, appear within 30 days.

After these results were obtained with spores selected at random, spores from the same ascus were used. The experiments were carried on with spores from one ascus in order to determine (1) whether or not the eight spores from a single ascus are of the same strain or whether both strains are represented; (2) if both strains are represented, whether four of the spores are of one strain and four of the other; and (3) whether the characteristics of the respective strains are segregated at the time of ascospore-formation.

It is difficult to secure all eight spores from one ascus. This was done by placing a rather thick coat of agar on a slide and then placing the slide about one centimeter above an apothecium which is discharging its spores. The slide is examined at frequent intervals in order to obtain the first ascospores that are discharged upon the agar. If too long an interval elapses between observations, spores become so thick on the slide that one can not distinguish with certainty the eight spores from a particular ascus. The spores are then picked off one by one and placed in separate petri dishes. Many attempts were made before all eight spores from a single ascus were



picked up, as many accidents happen, and even when the spores are obtained they may fail to germinate.

Finally seven spores out of eight from a single ascus were induced to germinate. The strains thus obtained were grown together in every possible combination as were those described above. The total number of combinations was 28. Strains 1, 2, 3, and 6 are sterile when placed together, as are the strains 4, 5, and 7, but a combination of any one of either group with any one of the other group produces a fertile culture. Strains when grown by themselves are always sterile.

An examination of table 2 will show that single-spore strains from the same ascus were found to be sterile when grown alone but in combination with certain other strains were fertile. This proved at once that strains derived from spores from the same ascus could produce apothecia, and that both strains were represented in the same ascus.

TABLE 2

Strains Used	No. Cultures	Results	
		Positive	Negative
1 + 1	6	0	6
1 + 2	6	0	6
1 + 3	12	0	12
1 + 4	10	10	0
1 + 5	12	12	0
1 + 6	12	0	12
1 + 7	12	12	0
2 + 2	6	0	6
2 + 3	12	0	12
2 + 4	10	10	0
2 + 5	6	6	0
2 + 6	11	0	11
2 + 7	12	11	1
3 + 3	6	0	6
3 + 4	6	6	0
3 + 5	6	6	0
3 + 6	11	0	11
3 + 7	10	10	0
4 + 4	10	0	10
4 + 5	10	0	10
4 + 6	6	6	0
4 + 7	12	0	12
5 + 5	6	0	6
5 + 6	9	9	0
5 + 7	11	0	11
6 + 6	6	0	6
6 + 7	6	6	0
7 + 7	6	0	6

If four of the spores represented one strain and three the other, then of the total number of combinations 12 should give positive and sixteen should give negative results. The table shows that this is what happened.

From these results it would seem that the genetic characters of the respective strains, at least in this species, are segregated at the time of ascospore-formation.

#### SUMMARY

1. A single spore or strain will not produce apothecia, but when two spores or strains, properly chosen, are grown together, apothecia are produced.

2. Apothecia are produced from spores from the same ascus. It is inferred that four of the spores represent plus strains and four minus strains.

3. The characters of the respective strains are segregated at the time of ascospore-formation.

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# A STUDY OF SUCTION FORCE BY THE SIMPLIFIED METHOD I. EFFECT OF EXTERNAL FACTORS

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## INTRODUCTION

Until recently only one osmotic quantity, the osmotic value at incipient plasmolysis, had been measured. In what is to follow, for the sake of brevity, I shall frequently use the term "osmotic value" instead of the above expression. By determining the incipient plasmolytic concentration, it had been established that  $\alpha$  mol. cane sugar possessed the same osmotic value as the cell sap at limit plasmolysis. Some authors properly termed this quantity the "osmotic value at incipient plasmolysis"; others named it "osmotic pressure," "turgor," "concentration of the cell sap," "suction force," etc.

In order to obtain a clear notion of the nature and importance of the various osmotic quantities, let us, with the aid of measurements made by Ursprung and Blum (22),<sup>1</sup> consider the same cell with its semipermeable plasmatic membranes, first in normal condition,<sup>2</sup> then at incipient plasmolysis, and finally when saturated with water.

By means of text figure 1 we may first study the cell sap. If cell sap is placed in an osmometer with semipermeable membrane, it will, under normal cell conditions, suck up water with a force of 9.7 atm.; in other words, the suction force of the content of the normal cell is 9.7 atm. At incipient plasmolysis this force rises to 10.5 atm., and, when the osmometer is water-saturated, it falls to 9.3 atm.

Let us turn our attention to wall pressure. At incipient plasmolysis this is naturally zero; when the cell is water-saturated, this pressure is necessarily equal to the suction force of the cell content, or 9.3 atm. Under normal conditions it has the intermediate value of 5.4 atm.

By *wall pressure* we mean the pressure exerted by the cell wall against the cell content; by *turgor pressure*, the pressure exerted by the cell content against the cell wall. In an isolated cell in a state of equilibrium, these two values are numerically equal (26).

We now turn our attention to the suction force of the cell, *i.e.*, the force with which the cell sucks up water. If we consider the isolated cell, it is evident that this force is the resultant of two components, which act in

<sup>1</sup> References are to literature cited in the bibliography appended to the second paper of this series, to appear in the following issue of the JOURNAL.




<sup>2</sup> The cell in its normal condition possesses the same volume as it does in the intact plant.

opposite directions: one, the suction force of the cell content, which tends to suck up water, and the other, the wall pressure, which tends to press out water. Hence, the suction force of the cell equals the suction force of the cell content minus the wall pressure; more concisely,

$$\text{Suction force of cell} = \text{Suction force of content} - \text{wall pressure.}$$

The corresponding values of this equation are given in text figure 1.

Until recently the osmotic value at incipient plasmolysis, or the suction force of the cell at incipient plasmolysis, was alone measured. In our case it is equal to 10.5 atm. Frequently false conclusions were drawn from these studies in incipient plasmolysis. For instance, the suction force of

	Normal Cell	Cell at Incipient Plasmolysis	Water Saturated Cell
			
Suction Force of Cell Content	9.7	10.5	9.3 Atm.
Wall Pressure	5.4	0.0	9.3 Atm.
Suction Force of Cell	4.3	10.5	0.0 Atm.

TEXT FIG. 1. The same cell under different conditions. The values of the various osmotic quantities, in atmospheres, are given below for each condition.

the above-described cell at incipient plasmolysis, 10.5 atm., was interchanged with the suction force of the normal cell, 4.3 atm.; thus an error of 6.2 atm. resulted. Again, the force which sucks up water was often interchanged with the pressure which forces it out. Frequently the following occurred: the osmotic value at incipient plasmolysis was *measured* (10.5 atm.) but was *called* turgor pressure (5.4 atm.); what was *meant*, in reality, was the suction force of the cell (4.3 atm.) (22).

Those who studied the movement of sap were interested in the suction force of the normal cell, those who studied the movement of pulvini were interested in turgor pressure; still, what was always measured was the osmotic value at incipient plasmolysis, which is entirely different from the values sought both in nature and in numerical expression.

The method of determining the *osmotic value at incipient plasmolysis* has been known for a long time; frequently, however, not enough attention has been paid to the choice of a proper plasmolyzing medium (26).

Theoretically the measuring of suction force is very simple; it is necessary to find only the concentration, say, of cane sugar in which the cell

keeps its normal volume (23, 20, 19). For information about the technical difficulties met with in trying to make such determinations, it is necessary to refer to original sources (23, 20, 19).<sup>3</sup> Under the heading "Methods," further details will be found of the new and decidedly simplified method, which can be used in the field, and which I myself followed in carrying out this work. More difficult, both in theory and in practice, is the measuring of wall pressure and turgor pressure. The formulae used for their determination, and the technical difficulties that are to be overcome, may also be sought in original sources (26, 23, 20, 19).

I intend to treat here of the results obtained in recent researches along the lines above suggested. The study of the osmotic value, which had practically been made only upon epidermal cells, was extended to other plant tissues (15); besides, a regular diurnal periodicity was discovered in all organs examined (16). To find the cause of this periodicity, the dependence of osmotic value upon exterior factors was studied (17, 1).

The suction force of the cell with normal volume, first of all, plays a rôle in the absorption of water from the soil by the roots. It is well known that soil holds water with a certain tenacity. The absorbing root cells must, therefore, overcome this resistance offered by the soil, if they are to take up enough water to allow the plant to thrive. The determination of this resistance is not easy. Experiments made in the past with osmometers, etc., could not give satisfactory answers; they presented only physical data, not the desired physiological solution. On the contrary, such resistance can be determined by measuring the suction force of the root hairs. This principle was first established theoretically and its correctness was then proven experimentally (24).

According to the opinion commonly received, the movement of water in the absorption zone of the root proceeds as follows: it is taken up by the root hairs, whence it wanders by means of osmotic suction from cell to cell through the cortex of the root to the water ducts. To test the correctness of this opinion, the suction force of the cells in the absorption zone of a root was measured. The results of this test are given in table I.

TABLE I. *Distribution of Suction Force in the Absorption Zone of the Root of Vicia Faba*

	Suction Force in Atm.
Epidermis.....	0.7
1st row of cortex cells.....	1.4
3d row of cortex cells.....	1.5
4th row of cortex cells.....	2.1
5th row of cortex cells.....	2.8
6th row of cortex cells.....	3.0
Endodermis.....	1.7
Wood parenchyma.....	0.9

<sup>3</sup> A further simplification can be found in formula 4, in the work of Ursprung and Blum (26, p. 27).

As table 1 shows, the suction force increases as we proceed from the epidermis to the innermost layer of cortical cells; at the endodermis, however, it falls much and suddenly (23); from here on, it decreases still more as we penetrate toward the vessels. The explanation of this "endodermal jump" is still to be studied more thoroughly (3).

Over and over again, in studies on the ascent of sap, an attempt was made to find an increase in suction force, proceeding from the base of a stem to its summit. That the result was a failure (4) was due, not to the nature of the matter, but to the use of wrong methods. What, for instance, Dixon measured, was not the suction force of the cell at all. In determining the real suction force of the leaves of a beech tree, the rule was established that the higher these leaves are located the higher is their suction force (19, 21). (See table 2.)

TABLE 2. *Suction Force of Beech Leaves at Different Heights*

Height	Upper Epidermis	Lower Epidermis	Palisades	Spongy Parenchyma
13.0 m.	10.5 atm.	9.9 atm.	17.1 atm.	14.3 atm.
11.1 m.	9.9 atm.	9.3 atm.	17.1 atm.	14.3 atm.
8.7 m.	9.3 atm.	8.4 atm.	15.6 atm.	12.4 atm.
2.7 m.	7.5 atm.	5.9 atm.	15.0 atm.	11.1 atm.

If, as is generally accepted, the water moves principally in the xylem of the vascular bundles, it is to be expected that, in transverse sections of the stem, the petiole, and the older portion of the root, a minimum suction force should exist in the xylem. Naturally there should be an increase in suction force in every tissue, proportional to the distance of the point of measurement from the vessels and to the distance along the direction of the flow of the sap. The results, recorded in table 3, agree with this view (20, 21). The absorption zone of the root forms the only exception, which is evident from what was said above (table 1).

TABLE 3. *Distribution of Suction Force in Root, Stem, and Petiole of the Ivy*

Transverse Section through	Hadrome (Xylem)	Pith	Inner Cortex	Outer Cortex	Phellogen	Epidermis
Petiole.....	8.1 atm.	8.4 atm.	8.4 atm.	9.0 atm.		9.3 atm.
Stem, 225 cm. high.....	4.2 atm.	4.8 atm.	5.0 atm.	7.3 atm.		7.4 atm.
Stem, 35 cm. high.....	2.1 atm.	2.4 atm.	2.9 atm.	3.4 atm.	3.7 atm.	
Root, older portion.....	2.1 atm.		2.4 atm.		3.2 atm.	
Root, absorption zone.....			1.6 atm.			1.0 atm.

Further explanations for the course taken by the ascending water were found in researches in the distribution of suction force in the leaf. An increase in suction force from the base to the apex was found in the midvein and the lateral veins of the ivy leaf. Especially interesting was the behavior of a row of palisade cells which extended from the midvein into a

portion of the leaf free from larger veins. From a minimum near the midvein, the suction force rose regularly, as table 4 shows, to the astonishingly high maximum of 32.6 atm., which was attained in the 210th palisade cell; from this point it fell (27).

TABLE 4. *Distribution of Suction Force in a Row of Palisade Cells in the Ivy Leaf*

Number of Palisade Cell	Suction Force in Atm.	Number of Palisade Cell	Suction Force in Atm.	Number of Palisade Cell	Suction Force in Atm.	Number of Palisade Cell	Suction Force in Atm.
3.....	12.1	60.....	18.1	132.....	28.4	207.....	32.1
11.....	13.3	72.....	20.4	145.....	29.7	210.....	32.6
21.....	13.7	84.....	22.7	155.....	30.2	214.....	32.6
30.....	15.0	99.....	25.1	167.....	30.7	223.....	32.1
44.....	17.1	113.....	26.0	190.....	31.6	230.....	32.1

In contrast to this regular rise in suction force, the osmotic value in the same row of palisade cells showed no regularity. This demonstrates nicely the important rôle played by suction force, and the unimportant one played by osmotic value, in supplying water to the plant.

A striking exception to the above-mentioned rule is afforded by the epidermis of the foliar lamina of the ivy. In a cross section of the leaf the suction force of the last-named tissue is not, as in the stem and petiole, the highest, but, on the contrary, is remarkably low.

TABLE 5. *Distribution of Suction Force in a Transverse Section of the Ivy Leaf*

Upper epidermis.....	8.0 atm.
Palisade cells.....	12.5 atm.
	12.2 atm.
	11.9 atm.
	9.8 atm.
Spongy parenchyma.....	10.1 atm.
	10.5 atm.
	10.8 atm.
Lower epidermis.....	7.3 atm.

As shown in table 5, the suction force increases from the innermost to the outermost palisade and spongy parenchyma layers; but instead of rising still further in the epidermis, it falls suddenly from 12.5 atm. to 8.0 atm., and from 10.8 atm. to 7.3 atm. (20, 21). This exception occurring in the epidermis of the leaf is important, both because of the characteristic manner in which the epidermis is supplied with water, and also because of its function as a water reservoir.

Two series of experiments are available in the study of wall pressure and turgor pressure (26). They refer, first, to the mechanism of the movements of the guard cells, and second, to the process of growth.

Measurements made upon the leaves of *Convallaria majalis* (table 6) showed that the stomatal aperture increases in width with turgor pressure. This was to be expected from the exhaustive studies made by Schwendener on the subject; still it was necessary to prove it experimentally.



TABLE 6. *Mean Turgor Pressure in the Guard Cells of Convolvallaria*

Mean Turgor Pressure	Corresponding Mean Width of Stomatal Apertures	Mean Turgor Pressure	Corresponding Mean Width of Stomatal Apertures
0-1 atm.....	0.4 $\mu$	4-5 atm.....	2.6 $\mu$
1-2 atm.....	1.0 $\mu$	5-8 atm.....	2.6 $\mu$
2-3 atm.....	1.6 $\mu$	8-10 atm.....	3.8 $\mu$
3-4 atm.....	2.1 $\mu$	10-16.8 atm.....	7.0 $\mu$

The relation between turgor and growth was less in harmony with what was to be expected. It may be rather generally accepted that the growth of a cell and its turgor, *i.e.*, the pressure exerted by the cell sap upon the cell wall, would rise and fall together. Actual measurements show that in a root the cells which are growing most vigorously in length (*ca.* 5 mm. distant from the tip) manifest, on the contrary, the least turgor. Comparing the turgor pressure with the other osmotic quantities, we find that the osmotic value increases as we approach the apex of the root, and hence bears no relation to growth. The suction force of the cell shows most clearly its highest value in that part of the root which is in the stage of maximum growth or is approaching this stage. According to researches made up to the present, the suction-force maximum seems to coincide with the elongation maximum. Hence, it is not probable that turgor pressure furnishes the necessary energy for the growth of the root cells. This, as also the lack of proportion existing between turgor tension and growth in length, contradicts the Sachs-De Vries theory of growth. The high suction force in the elongating zone is easily understood, since growth means an increase in volume, which latter depends principally upon the absorption of water.

TABLE 7. *Osmotic Value, Suction Force, and Turgor Pressure of the Root of Vicia Faba*

Tissue Examined	Distance from Growing Point in Mm.	Osmotic Value * in M Cane Sugar	Suction Force in Atm.	Turgor Pressure in Atm.
Epidermis or cells of outer cortex...	1.5	0.50	7.4	3.9
	3.0	0.49	7.7	4.0
	5.0	0.47	10.0	2.7
	8.0	0.45	0.4	9.4

\* Osmotic value at incipient plasmolysis.

Again and again experiments were unsuccessfully made on geotropically curved root tips to find a relation existing between growth and osmotic value (26). As in the straight root, so also in the curved, the supposed differences become evident if the proper osmotic quantities are measured. The suction force in the curved radicle of the *Vicia Faba* seedling (table 8) showed itself much higher on the more vigorously growing convex side than on the concave side; whereas the turgor pressure showed the contrary

values. Here, again, the greater increase in volume accompanying the stronger growth accounts satisfactorily for the high suction force of the convex side.

TABLE 8. *Curving Radicle of Vicia Faba*

Tissue Examined	Side	Osmotic Value * in M Cane Sugar	Suction Force in Atm.	Turgor Pressure in Atm.
Outer Cortical layers }	Concave	0.40	0.5	8.0
	Convex	0.38	8.2	1.3

\* Osmotic value at incipient plasmolysis.

Coinciding with the conditions in the curving root were those existing in the curved pulvinus of *Tradescantia* (26); the higher suction force and the lower turgor pressure were found on the more strongly growing convex side (table 9). While in the curved root the convex side shows values similar to those of the corresponding zone in the straight root, in the pulvinus the concave side shows them. This, no doubt, is due to the fact that the straight organ of the pulvinus is more or less fully grown, while the straight zone of the root is still in its period of greatest elongation. In the curved pulvinus it was the concave side, in the curved root the convex side, which showed about the same conditions as the corresponding portion of the straight organ.

TABLE 9. *Straight and Curving Pulvinus of Tradescantia*

Tissue Examined	Side	Osmotic Value * in M Cane Sugar	Suction Force in Atm.	Turgor Pressure in Atm.
Pith.....	Straight	0.31	2.7	3.8
	Concave	0.31	2.7	4.6
	Convex	0.32	7.0	1.1
Starch sheath...	Straight	0.32	3.6	4.1
	Concave	0.34	4.1	3.6
	Convex	0.31	7.1	0.4
Cortex.....	Straight	0.30	2.5	3.8
	Concave	0.31	3.2	4.7
	Convex	0.31	7.0	0.6

\*Osmotic value at incipient plasmolysis.

#### METHODS

My own research confines itself to the measurement of suction force. The method mentioned in the introduction permits the determination of the suction force of individual cells. This, however, often presents great technical difficulties and hence would not have allowed of the measurement of a larger number of species in a reasonable length of time.

It seemed desirable to direct the research to problems of wider range, e.g., the measurement of the suction force of plants of diverse habitats, various modes of life, and different positions in the systematic order.

By the discovery of a simpler method (25), which could be used in the field and which allowed more rapid work, this new series of experiments was made possible. My measurements, described in the following pages, are a first step in this direction. I proposed to measure as many wild plants, and these of as varied habitats as possible, in the immediate vicinity of the Freiburg Botanical Institute. During the winter months I studied, in the laboratory, the effect of external factors on suction force; unfortunately, however, unforeseen circumstances made it necessary to do this somewhat summarily.

Here I wish to express my heartfelt thanks to Prof. A. Ursprung of the University of Freiburg, Switzerland, who by his continual interest and suggestions made this work possible.

I used volumetrically normal cane sugar solutions, (1 *M*, 342 g. cane sugar in 1000 cc. solution). The 1 *M*, 1½ *M*, and 2 *M* base solutions, made in measuring flasks, were diluted by means of Schellbach burettes. These dilutions differed by 0.05 *M* from one another. These concentrations were then placed in small glass bottles of 20 cc. content, firmly closed with ground-glass stoppers. Each solution was used only four times.

The plant organs, or portions of organs, to be examined, after having been cut from the plant, were immediately placed in paraffin oil. In the laboratory, appropriate strips of tissue were cut from the above-described material under paraffin oil. These strips were so trimmed that their lengths could be exactly determined.

In order to determine these lengths, I placed each strip on a slide on which a distance of 5 cm. was subdivided into whole or half millimeters. The strips lay in paraffin oil, covered with a cover slip. By means of proper microscopic enlargement, I first determined the number of whole or half millimeters and the remainder in eye-piece micrometer divisions. By means of filter paper, the paraffin oil was rapidly removed, and the strips were then placed in the small bottles containing sugar solutions, where they remained for one half to one and one half hours. Next, the lengths were again measured in the given sugar solutions and note was taken of their variations in length. In some few cases the objects to be examined were so small that all measurements were made by means of the eye-piece micrometer. This was the case, for instance, for *Angelica sylvestris*.<sup>4</sup>

The percentage of error of the readings was always less than 0.4. Errors caused by parallax, which readily occurred with thicker tissues, were sufficiently reduced by proper cutting. With material that was very delicate, as, for instance, roots, pistils, stamens, etc., greater errors were avoided by supporting the cover slip with particles of glass. Table 10 shows how the average suction force of these strips of tissue was determined.

<sup>4</sup> The complete name (including the name of the author) of each species used in my field experiments is given in the second paper of this series.

TABLE 10

Concentration of Cane Sugar ( <i>M</i> )	Variation % of Original Lengths of Strips after Having Been Carried from Paraffin Oil into Cane Sugar Solutions			
	<i>Borago officinalis</i> Corolla	<i>Trifolium pratense</i> Corolla	<i>Elodea canadensis</i> Root Tip	<i>Zea Mays</i> Leaf
0.05.....			+ 0.9	
0.10.....	+ 2.8		+ 0.7	+3.8
0.15.....	+ 1.3		- 1.7	+2.4
0.20.....	0.0	+1.6	- 4.6	+0.8
0.25.....	- 0.4	+1.0	-12.0	-1.6
0.30.....	- 2.8	+0.5		-4.0
0.35.....	- 6.4	0.0		-6.1
0.40.....	- 6.6	-0.3		-8.0
0.45.....	- 8.7	-0.5		
0.50.....	-11.0	-0.5		
0.55.....	-11.6			
0.60.....	-13.0			

The suction force of the corolla of *Borago officinalis* is equivalent to 0.20 *M* cane sugar solution, which equals 5.3 atm.; that of *Trifolium pratense*, 0.35 *M* or 9.6 atm. However, the suction force of the root tip of *Elodea canadensis* lies between 0.10 *M* and 0.15 *M* (3.2 atm.), while that of the leaf of *Zea Mays* lies between 0.20 and 0.25 *M* (5.9 atm.). The intermediate values were found by interpolation.

As will be seen from table 10, the variation of the strips was not always directly proportional to the sugar concentrations. It could not be taken for granted that the variations in dimensions would be proportional to the variations in concentration employed, since the venation is different, and for this reason alone the reactions might be different.

Only such suction-force determinations will be found in this work as are based upon a larger number of individual measurements (from 4 to 10); such are those in table 10.

The strips of tissue were cut of such length as to allow a maximum of reaction. The sections were, therefore, generally cut perpendicularly to the veins; but at times the material to be examined was such as to render this method impractical. This was commonly the case with the ray flowers of the Compositae, which were cut parallel to the veins. Larger veins were always avoided.

Since I was frequently obliged to bring aquatic plants to the laboratory from great distances, I had occasionally to keep them in paraffin oil for as long a time as one hour. It was, therefore, essential to know whether or not the suction force of these specimens fell or rose during their stay in oil. To be certain of this, I tested the corolla of *Nymphozanthus luteus*, leaves of *Elodea canadensis*, and root tip of *Potamogeton crispus*. As will be seen from table 11, even after the last two had remained in oil 48 hours, and the first 120 hours, there was no perceptible change.

TABLE II

Name	Organ Examined	Length of Time in Paraffin Oil	Suction Force in Atm.	Difference of Suction Force in Atm.
<i>Elodea canadensis</i> . . . . .	Leaf . . . .	Fresh . . . . .	4.8	
		In paraffin oil 48 hrs. . . . .	4.0	— 0.8
<i>Potamogeton crispus</i> . . . . .	Root tip	Fresh . . . . .	4.0	
		In paraffin oil 48 hrs. . . . .	3.5	— 0.5
<i>Nymphozanthus luteus</i> . . . . .	Corolla . .	Fresh . . . . .	4.8	
		In paraffin oil 120 hrs. . . . .	4.0	— 0.8

In order to find the equivalent in atmospheres for each sugar concentration given in terms of molecular solutions, I used the tables of Ursprung and Blum (18), as also those of Ursprung and Hayoz (27). These tables had been recently extended by the first-mentioned authors from 0.96 *M*

TABLE 12

<i>M</i> Cane Sugar in 1 L. Solution	Osmotic Pressure at 20° in Atm.	<i>M</i> Cane Sugar in 1 L. Solution	Osmotic Pressure at 20° in Atm.	<i>M</i> Cane Sugar in 1 L. Solution	Osmotic Pressure at 20° in Atm.
0.01	0.3	0.41	11.4	0.81	26.0
0.02	0.5	0.42	11.7	0.82	26.4
0.03	0.8	0.43	12.1	0.83	26.8
0.04	1.1	0.44	12.4	0.84	27.2
0.05	1.3	0.45	12.7	0.85	27.6
0.06	1.6	0.46	13.0	0.86	28.0
0.07	1.9	0.47	13.3	0.87	28.4
0.08	2.1	0.48	13.7	0.88	28.8
0.09	2.4	0.49	14.0	0.89	29.3
0.10	2.6	0.50	14.3	0.90	29.7
0.11	2.9	0.51	14.6	0.91	30.2
0.12	3.2	0.52	15.0	0.92	30.7
0.13	3.4	0.53	15.3	0.93	31.1
0.14	3.7	0.54	15.6	0.94	31.6
0.15	4.0	0.55	16.0	0.95	32.1
0.16	4.2	0.56	16.3	0.96	32.6
0.17	4.5	0.57	16.7	0.97	33.1
0.18	4.7	0.58	17.1	0.98	33.6
0.19	5.0	0.59	17.4	0.99	34.1
0.20	5.3	0.60	17.8	1.00	34.6
0.21	5.6	0.61	18.1	1.01	35.1
0.22	5.9	0.62	18.5	1.02	35.7
0.23	6.1	0.63	18.9	1.03	36.2
0.24	6.4	0.64	19.2	1.04	36.7
0.25	6.7	0.65	19.6	1.05	37.2
0.26	7.0	0.66	20.0	1.06	37.7
0.27	7.3	0.67	20.4	1.07	38.2
0.28	7.5	0.68	20.7	1.08	38.8
0.29	7.8	0.69	21.1	1.09	39.3
0.30	8.1	0.70	21.5	1.10	39.8
0.31	8.4	0.71	21.9	1.11	40.4
0.32	8.7	0.72	22.3	1.12	40.9
0.33	9.0	0.73	22.7	1.13	41.5
0.34	9.3	0.74	23.1	1.14	42.0
0.35	9.6	0.75	23.4	1.15	42.5
0.36	9.9	0.76	23.8	1.16	43.1
0.37	10.2	0.77	24.3	1.17	43.7
0.38	10.5	0.78	24.7	1.18	44.2
0.39	10.8	0.79	25.1	1.19	44.8
0.40	11.1	0.80	25.5	1.20	45.4

TABLE 12 (continued)

<i>M</i> Cane Sugar in 1 L. Solution	Osmotic Pressure at 20° in Atm.	<i>M</i> Cane Sugar in 1 L. Solution	Osmotic Pressure at 20° in Atm.	<i>M</i> Cane Sugar in 1 L. Solution	Osmotic Pressure at 20° in Atm.
1.21	46.0	1.61	74.8	2.01	117.8
1.22	46.6	1.62	75.7	2.02	119.1
1.23	47.2	1.63	76.5	2.03	120.5
1.24	47.8	1.64	77.4	2.04	121.8
1.25	48.4	1.65	78.3	2.05	123.1
1.26	49.0	1.66	79.2	2.06	124.4
1.27	49.6	1.67	80.2	2.07	125.8
1.28	50.3	1.68	81.2	2.08	127.2
1.29	50.9	1.69	82.1	2.09	128.7
1.30	51.6	1.70	83.0	2.10	130.1
1.31	52.2	1.71	84.0	2.11	131.5
1.32	52.9	1.72	85.0	2.12	133.0
1.33	53.6	1.73	86.0	2.13	134.4
1.34	54.3	1.74	87.0	2.14	135.9
1.35	54.9	1.75	88.0	2.15	137.3
1.36	55.6	1.76	89.0	2.16	138.7
1.37	56.3	1.77	90.1	2.17	139.8
1.38	57.0	1.78	91.1	2.18	141.3
1.39	57.7	1.79	92.2	2.19	142.8
1.40	58.4	1.80	93.2		
1.41	59.1	1.81	94.3		
1.42	59.9	1.82	95.4		
1.43	60.6	1.83	96.5		
1.44	61.3	1.84	97.6		
1.45	62.1	1.85	98.7		
1.46	62.8	1.86	99.9		
1.47	63.6	1.87	101.0		
1.48	64.3	1.88	102.2		
1.49	65.0	1.89	103.3		
1.50	65.8	1.90	104.5		
1.51	66.5	1.91	105.7		
1.52	67.3	1.92	106.9		
1.53	68.1	1.93	108.1		
1.54	68.9	1.94	109.2		
1.55	69.7	1.95	110.3		
1.56	70.6	1.96	111.5		
1.57	71.5	1.97	112.7		
1.58	72.5	1.98	114.0		
1.59	73.1	1.99	115.2		
1.60	73.9	2.00	116.6		

to 2.19 *M* cane sugar solutions. I insert a complete table (table 12) ranging from 0.01 *M* to 2.19 *M* cane sugar solutions, with their equivalents in atmospheres.

#### THE EFFECTS OF EXTERNAL FACTORS ON SUCTION FORCE

Although our knowledge of the effects of external factors on suction force is slight, I present these effects first because they form the basis for an understanding of what is to follow.

#### Moisture of the Soil

The effect of the moisture of the soil was studied by observations made in the field as well as in the laboratory.

TABLE 13. Effect of Rain on Suction Force

Name of Plant	Organ Examined	Location	Date, 1923	Rainfall *	Suction Force in Atm.	Variation of Suction Force in Atm.
<i>Campanula Rapunculus</i> .....	Corolla	Wood	7/17, 4 P.M.	Before rain	13.7	
	Corolla	Wood	7/18, 8 A.M.	After 14.2 mm. rain	6.1	7.6
<i>Potentilla anserina</i> .....	Corolla	Wood	7/17, 10:30 A.M.	Before rain	8.1	
	Corolla	Wood	7/18, 8 A.M.	After 14.2 mm. rain	6.1	2.0
<i>Trifolium pratense</i> .....	Corolla	Wood	8/24, 8 A.M.	Before rain	11.1	
	Corolla	Wood	8/25, 8 A.M.	After 4.6 mm. rain	7.5	3.6
	Corolla	Wood	8/27, 8 A.M.	Before rain	9.5	
	Corolla	Wood	8/28, 8 A.M.	After 5.8 mm. rain	6.5	3.0

\* All rainfall data were taken from tables of the Meteorological Station of Freiburg, Switzerland. I here take the opportunity to thank Prof. A. Gockel for his kindness in placing these tables at my disposal.

TABLE 14. Effect of Rain on Suction Force

Name of Plant	Organ Examined	Location	Date, 1923	Rainfall	Suction Force in Atm.	Variation in Suction Force in Atm.
<i>Semperivium tectorum</i> .....	Foliar lamina	Alpine rock cleft	7/31, 2 P.M.	Before rain	ca. 45.5	18.0
	Foliar lamina	Alpine rock cleft	8/1, 3 P.M.	After heavy rain	ca. 27.5	
<i>Satureia alpina</i> .....	Foliar lamina	Alpine rubble	7/30, 11 A.M.	Before rain	34.5	21.0
	Foliar lamina	Alpine rubble	8/1, 11 A.M.	After heavy rain	13.5	
	Corolla	Alpine rubble	7/30, 11 A.M.	Before rain	12.0	
	Corolla	Alpine rubble	8/1, 11 A.M.	After heavy rain	8.0	4.0
<i>Geranium sylvaticum</i> .....	Foliar lamina	Alpine rubble	7/31, 6 P.M.	Before rain	21.5	
	Foliar lamina	Alpine rubble	8/1, 6 P.M.	After heavy rain	21.5	0.0

### Field Observations

Table 13 presents results drawn from observations made on one and the same plant examined at short intervals, before and after a rain.

More marked differences were found by G. Blum in the Freiburg Pre-alps as table 14 shows (14).

Tables 13 and 14 show clearly what great suction-force variations are possible after a heavy rain, especially if it follows a long period of drought. Also noticeable is the varying reaction of different species in one and the same place. Thus, the corolla of *Campanula Rapunculus* showed a drop of 7.6 atm. at the same time that the similar organ of *Potentilla anserina* showed one of 2.0 atm. Still more striking is the example in which the foliar lamina of *Satureia alpina* showed a variation of 21 atm. while the leaf blade of *Geranium sylvaticum* showed no variation at all. It is also possible that different organs of the same plant react differently. In this way, as shown in the table, the foliar lamina of *Satureia alpina* varied 21 atm. at the same time that the corolla of the same plant changed 4 atm.

### Laboratory Observations

In these and all following measurements of *Chrysanthemum frutescens*, different ray flowers of the same capitulum were compared. To determine whether this was permissible, I first tested the suction force of various ray flowers on the same head and found it equal in all such flowers of the same capitulum, e.g.:

Suction force of florets on one side of the capitulum..... 4.6 atm.  
Suction force of florets on the opposite side of the same capitulum..... 4.6 atm.

A pot culture of the above-mentioned plant was not watered for 8 days. The soil, which had become extremely dry, was then thoroughly soaked. Table 15 shows that the suction force fell in one hour 5.1 atm., and that after 7 hours it had dropped from 19.1 atm. to 6.9 atm.

TABLE 15. *Chrysanthemum frutescens*

Time	Number Hrs. after Being Moistened	Suction Force of Ray Flower in Atm.	Variation of Suction Force in Atm.
10 A.M.....	(Start) 0	19.1	0.0
11 A.M.....	1	14.0	5.1
12 A.M.....	2	11.8	7.3
1 P.M.....	3	7.4	11.7
5 P.M.....	7	6.9	12.2
8 A.M.....	22	4.7	14.4

Since not only the soil and roots, but also the portions of the plant above ground, are moistened by a rain, it was of interest to see what effect would be produced when the underground organs were not wetted, while those above ground were. With this problem in view, the pot in which grew a healthy *C. frutescens* was carefully protected against moisture in a closed



glass jar, and the plant was then exposed to an artificial rain. Comparing table 16 with table 15, it will be seen that the sprinkling of only the portions of the plant above the soil produced no appreciable difference in suction force.

TABLE 16. *Chrysanthemum frutescens*

Time	Number of Hrs. Exposed to Artificial Rain	Suction Force of Ray Flowers in Atm.	Variation of Suction Force in Atm.
10 A.M.....	(Start) 0	12.4	
11 A.M.....	1	12.7	+ 0.3
12 A.M.....	2	11.3	- 1.1
1 P.M.....	3	12.6	+ 0.2
5 P.M.....	7	11.7	- 0.7

In tables 35 and 36, opposite methods show opposite results to those given in table 15. Potted *C. frutescens* plants, whose soil was thoroughly watered, and which, therefore, had low suction forces, were given no water during the experiments. The suction force increased in practically every case.

According to these data the suction force falls when the soil of the potted *C. frutescens* is watered. If water is given in different quantities to several specimens, it is to be expected that those plants receiving less water will manifest higher suction force.

The soil of four potted specimens of *Bellis perennis* was thoroughly watered, and the suction force of a leaf of each plant determined. The mean suction force of these was 7.5 atm. (Preparatory experiments had already shown that equally developed leaves of a given *Bellis* plant manifested no noteworthy individual differences.) After three or four days, when the soil was slightly drier, varying quantities of water were given to the plants, daily, during a period of two weeks. Next the suction force of a leaf of each plant was again measured. The results, though not so marked as might be expected, agree with my surmise. They are shown in table 17.

TABLE 17. *Bellis perennis*

Amount of Water Given Daily to Individual Plants for a Period of 2 Weeks	Variation in Suction Force of the Leaves of each <i>Bellis perennis</i> Plant after Receiving the Given Amounts of Water Daily for 2 Weeks
150 g.....	+ 1.37 atm.
75 g.....	+ 2.40 atm.
50 g.....	+ 3.81 atm.
5 g.....	+ 4.20 atm.

The great importance of soil moisture became evident in experiments with cane sugar solution cultures of *Bellis perennis* and *Zea Mays*.

The roots of different *Bellis perennis* plants were placed in distilled water for five or six days, after which the suction force of a leaf of each plant was determined. Then the distilled water was replaced by cane sugar solutions of various concentrations. After the first and after the

ninth day the cultures were measured. The results are given in table 18. The average suction force of all plants tested in the water cultures at the beginning of the experiment had been 10 atm.

TABLE 18. *Bellis perennis*

Suction Force of Cane Sugar Solutions	Suction Force of Leaves of <i>Bellis perennis</i> Varied	
	After 1 Day	After 9 Days
0.0 atm.....	−0.3 atm.	− 2.0 atm.
2.6 atm.....	+ 0.5 atm.	—
5.3 atm.....	+ 2.5 atm.	+ 8.2 atm.
8.1 atm.....	+ 1.2 atm.	+10.5 atm.
9.6 atm.....	+ 6.3 atm.	+14.2 atm.
11.1 atm.....	+ 7.9 atm.	Dead

In the next experiments and in all others made by me with *Zea Mays*, in order to determine the effect of external factors on its suction force, the measurements at the start and at the end were always made on the same leaf of the same plant. At the start I always tested the upper half, at the end of the experiment the lower half of the leaf.

Preparatory experiments had established that the upper and lower halves of the same leaf had the same suction force, if care was taken to cut leaf material as near as possible to the division line between the upper and lower halves of the leaf.

TABLE 19

	Suction Force in Atm.
Upper half of <i>Zea Mays</i> leaf.....	4.0
Lower half of same <i>Zea Mays</i> leaf.....	4.0

In another preparatory experiment I measured the upper halves of leaves. Then, in some cases, I applied vaseline to the exposed cells of the lower remaining and attached halves of the same leaves; in others, I applied no vaseline. After allowing these lower halves to continue in this state for periods varying for from 4 hours to 13 days, I measured them. Naturally the cut cells, in immediate contact with the air, dried out. When the suction-force measurements were made, these cells were first removed by cutting off a transverse strip of 2 or 3 millimeters. In all cases, the suction-force was practically equivalent to that which I had determined before in the upper halves of the same leaves. This equivalence is naturally reckoned on comparison with controls. Control plants were used in all experiments, since cultures kept in the laboratory showed slight diurnal variations, similar to those of plants growing in the open (see the following paper of this series).

The suction force of the upper halves of young primary leaves of a number of *Zea Mays* cultures in distilled water was measured, and then the plants from which these cuttings had been taken were placed in cane

sugar solutions of varying concentrations. They were kept there 3, 6, 9, and 13 days respectively, after which the suction force of the lower halves of the given leaves was measured. The variations in suction force, as compared with that of plants kept in distilled water, are shown in table 20. Before these experiments the average suction force of all the plants was approximately 6 atm.

TABLE 20. *Zea Mays*

Suction Force of Cane Sugar Solution	Suction Force of Leaves Increased as Compared with Control Plants			
	After 3 Days	After 6 Days	After 9 Days	After 13 Days
2.6 atm.....	0.2 atm.	0.7 atm.	3.6 atm.	Dead
5.3 atm.....	1.4 atm.	2.3 atm.	Dead	Dead
8.1 atm.....	2.3 atm.	3.9 atm.	Dead	Dead
9.6 atm.....	5.6 atm.	4.1 atm.	Dead	10.0 atm.

### Humidity of the Air

#### Field Observations

I had noticed repeatedly that the same organ of a plant showed a higher suction force toward noon than early in the morning and late in the evening. A number of these cases are given in table 21. That the suction-force variations here shown really were caused by the humidity of the atmosphere will appear later.

Equal fluctuations in the atmospheric moisture do not always produce like effects on the suction force. This will be seen from the observations shown in table 22 (14). In this table the measurements made on days when there were similar air-humidity fluctuations are joined by braces.

TABLE 22. *Equal Atmospheric Humidity Fluctuations Producing Different Effects upon Suction Force*

Date, 1923	Daily Variation		Amount of Rainfall in Mm. on Day of Observation and on 2 Preceding Days
	of Rel. Air Humidity, %	of Suction Force of <i>Bellis perennis</i> Corolla in Atm.	
{ 10/4 .....	24	1.4	17.9
{ 9/26 .....	24	3.8	0.1
{ 8/10 .....	52	8.0	0.0
{ 9/19 .....	52	3.9	7.3

Equal fluctuations of air humidity (24 %) produced changes in suction force of 1.4 atm. in one case, and 3.8 atm. in the other. In like manner, with a fluctuation of atmospheric moisture of 52 %, there were different suction-force variations, viz., 3.9 atm. and 8.0 atm.

A glance at the rain column of table 22 shows that there is a small change in suction force when the soil is very moist, and a relatively large change when it is dry.

TABLE 21

Name of Plant	Location	Date, 1923	Rel. Air Humidity, %	Temp., ° C.	Weather*	Organ Examined	Suction Force in Atm.
<i>Helleborus foetidus</i> .....	Edge of wood	† { 5/14, 9:30 A.M. 5/14, 3 P.M.	70 60	14 16	Cloudy Cloudy	Lower foliar epidermis over midvein near apex, of same leaflet	10.5 11.0
	Edge of wood	{ 5/15, 9 A.M. 5/15, 4 P.M.	76 60	9 14	Cloudy Cloudy	Lower foliar epidermis over middle portion of midvein of middle leaflet	9.5 10.5
<i>Epilobium angustifolium</i> .	Protected wood	{ 7/10, 8 A.M. 7/10, 4:15 P.M.	73 35	23 29	Bright Bright	Corolla	6.0 8.0
<i>Linaria minor</i> .....	Railroad track	{ 9/ 6, 9:30 A.M. 9/ 6, 2:45 P.M.	85 60	14 18	Bright Bright	Lower lip of corolla	9.5 10.5
	Edge of wood	{ 9/11, 8 A.M. 9/11, 1:30 P.M.	80 53	15 23	Bright Bright	Standard of corolla	12.0 10.5
<i>Trifolium pratense</i> .....	Roadside	{ 9/12, 8 A.M. 9/11, 8 A.M. 9/11, 1:30 P.M.	80 80 53	15 15 23	Bright Bright Bright	Standard of corolla Standard of corolla Standard of corolla	9.5 11.0
<i>Solanum nigrum</i> .....	In running water before molasse wall	{ 9/28, 9:30 A.M. 9/28, 2:30 P.M.	43 40	14 19	Cloudy Cloudy	Corolla	6.5 8.0
	Roadside	{ 9/28, 9:30 A.M. 9/28, 2:30 P.M.	43 40	14 19	Cloudy Cloudy	Corolla Corolla Corolla	5.5 6.0

\* Practically all meteorological data contained in this work were gathered by me in observations made outside of a north window of the laboratory. When this was not the case, the fact is noted. The window overlooked the locality where I found most of my plants. Naturally, for experiments in the laboratory all data were taken at my table.

† The brace indicates that the measurements in question were made on one and the same plant.

### Laboratory Observations

A number of experiments were performed by C. Hayoz with water cultures of *Zea Mays*, as well as with potted *Bellis perennis* plants. The results follow:

*Water Cultures.* Young *Zea Mays* plants, raised in tap water, were transferred from laboratory air (relative air moisture of 70 %, suction force of leaf 5.3 atm.) to bell jars with 100 % and 20 % ( $\text{CaCl}_2$ ) air moisture,<sup>5</sup> where they remained four hours. No change in suction force was noted. In my own experiment, however, in which I exposed *Zea Mays* to different air moistures for a period of 48 hours, a variation in suction force occurred (table 23).

TABLE 23. *Zea Mays*

Relative Air Humidity, %	Length of Time Exposed	Suction Force of Leaf in Atm.
70.....		5.3
100.....	4 hrs.	5.3
20.....	4 hrs.	5.3
50.....		5.0
100.....	48 hrs.	4.5
14.....	48 hrs.	7.1

*Potted Plants with Moist Soil.* In this experiment *Bellis perennis* plants were used, similar to those which were employed by Kandija (7) in studying periodicity.

During the first part of the experiment the plants were left uncovered; then pot and soil were covered with tin foil in order to prevent the soil's giving off or taking up moisture (table 24).

TABLE 24. *Bellis perennis*

Uncovered Pot			Pot and Soil Covered with Tin Foil		
Rel. Air Humidity, %	Length of Time Exposed	Suction Force of Leaf in Atm.	Rel. Air Humidity, %	Length of Time Exposed	Suction Force of Leaf in Atm.
Start 70.....		6.7	(Start) 100..		6.7
Same leaf { 100.....	3 hrs.	6.7	32..	3 hrs.	8.1
60.....	3 hrs.	9.6			
Same leaf { 60.....	3 hrs.	9.6	32..	3 hrs.	8.1
100.....	3 hrs.	6.7	100..	3 hrs.	6.7

Uncovered pot: A variation of air moisture of 40 % causes a suction force fluctuation of 2.9 atm.

Covered pot: A variation of air moisture of 68 % causes a suction force fluctuation of only 1.4 atm.

<sup>5</sup> To create a 100 % humid atmosphere in this, as well as in the following experiments, where this condition is demanded, a bell jar was used, partly lined on the inside with filter paper which dipped in a plate filled with water. Neither the pot nor the plant came in contact with the water or with the filter paper. A dry atmosphere was created by placing the plant in a bell jar which contained  $\text{CaCl}_2$  above the plant in a screen cage, and below in an appropriate dish.

*Potted Plants with Fairly Dry Soil* (table 25). (The same as used with moist soil.)

TABLE 25. *Bellis perennis*

Uncovered Pot			Pot and Soil Covered with Tin Foil			
Rel. Air Humidity, %	Length of Time Exposed	Suction Force of Leaf in Atm.	Rel. Air Humidity, %	Length of Time Exposed	Suction Force of Leaf in Atm.	
Same leaf { 100.... 60....	4 hrs.	8.1	100.....	4 hrs.	5.3	} Same leaf
	4 hrs.	14.3	35.....	4 hrs.	7.3	
Another pot { 100.. 60..	1 hr.	7.0	100.....	1 hr.	9.6	} Same leaf
	1 hr.	14.3	35.....	1 hr.	11.1	

Uncovered pot: A variation of air moisture of 40 % causes a suction-force fluctuation of up to 7.3 atm.

Covered pot: A variation of air moisture of 65 % causes a suction-force fluctuation of up to 2.0 atm.

From these experiments we may draw the following conclusions:

1. With increasing air moisture, the suction force of the leaf falls; with decreasing air moisture, it rises.

2. Like fluctuations of air moisture influence the suction force of the leaf thus: the drier the soil the greater the variation.

3. The suction force is influenced less directly by air moisture than indirectly; *i.e.*, the atmospheric humidity acts, not directly on the leaf, but principally indirectly by varying the moisture of the soil.

This conclusion is based on the experiments whose results are shown in tables 24 and 25. A potted plant is growing in comparatively dry soil, the pot and soil are covered with tin foil and exposed to various air moisture; the suction force varies little. Thus, in the last example of the experiment recorded in table 25, the suction force varied by 1.5 atm. instead of 7.3 atm., although the air moisture varied much more (65 % and 40 %), and although the soil was much drier than in the preceding parallel experiment with the uncovered pot.<sup>6</sup>

### Temperature

That low temperatures render the absorption of water by plants more difficult was already known. Pfeffer (8), for example, says that "tobacco plants in pots begin to flag as soon as the temperature of the moist soil falls to from 2° C. to 4° C." Hence, a rise in suction force was to be expected with a lowering of soil temperature. This was inferred by Ursprung and Blum (24), who, during a period of 30 hours, slowly lowered the temperature of the soil of potted *Vicia Faba* seedlings from 18° C. to 2° C. The result was that the suction force of the roots rose from 0.7 atm. to 1.9 atm.

<sup>6</sup> Regarding the relation between saturation deficit and suction force, refer to the following paper in this series, section on "Periodic Variations."

## Field Observations

In winter, when air and soil temperatures were low, the suction force of *Helleborus foetidus* and *Vinca minor* rose in a most significant manner (table 26). During the week preceding the February measurements, the temperature of the air often fell to  $-10^{\circ}\text{C}$ . and the soil was solidly frozen. At the end of May and the beginning of June, the temperature of the air was always above  $0^{\circ}\text{C}$ ., and at times was higher than  $20^{\circ}\text{C}$ .

TABLE 26. *Effect of Temperature on Suction Force*

Name of Plant	Organ Examined	Location	Date	Air Temp.	Suction Force
<i>Helleborus foetidus</i> ..	Perianth	Edge of wood	5/30/23, 9 A.M.	+ $9^{\circ}\text{C}$ .	10.5 atm.
<i>Helleborus foetidus</i> ..	Perianth	Edge of wood	2/25/24, 8 A.M.	- $2^{\circ}\text{C}$ .	51.5 atm.
<i>Vinca minor</i> .....	Lower foliar epidermis	Edge of wood	6/ 8/23, 2 P.M.	+ $18^{\circ}\text{C}$ .	7.5 atm.
<i>Vinca minor</i> .....	Lower foliar epidermis	Edge of wood	2/27/24, 8 A.M.	+ $2^{\circ}\text{C}$ .	23.5 atm.

The problem, therefore, was to determine which factor, soil temperature or air temperature, was more largely responsible for the rise in suction force and to what degree. Clues to the solution of this problem are found in my observations given in table 27. During the winter I measured two submersed aquatic plants, *Potamogeton crispus* and *Elodea canadensis*, which had been fished out of a pond covered with four centimeters of ice.

TABLE 27. *Effect of Temperature on Suction Force*

Name of Plant	Organ Examined	Location	Date	Air Temp.	Suction Force in Atm.
<i>Potamogeton crispus</i> ...	Foliar lamina	Fish pond	6/27/23, 9 A.M.	$13^{\circ}\text{C}$ .	6.0
<i>Potamogeton crispus</i> ...	Foliar lamina	Fish pond	2/28/24, 8 A.M.*	- $6.7^{\circ}\text{C}$ .	4.5
<i>Elodea canadensis</i> .....	Foliar lamina	Fish pond	6/20/23, 9 A.M.	$15^{\circ}\text{C}$ .	4.5
<i>Elodea canadensis</i> .....	Foliar lamina	Fish pond	2/28/24, 8 A.M.*	- $6.7^{\circ}\text{C}$ .	4.0

\* Water temperature at  $0^{\circ}\text{C}$ .

It is very remarkable that in winter the suction force of *Potamogeton crispus* and *Elodea canadensis* is not higher, but actually lower, than in summer. This seems to point to the fact that in winter the high values for land plants are caused less by the low leaf temperatures than by the increased difficulty presented to water-absorption by the frozen soil. Of course it is necessary to note that the intercellular spaces of the perianth of *Helleborus foetidus* and the leaf of *Vinca minor* contained ice crystals, while this was not the case with the leaves of the aquatic plants examined.

By remaining in paraffin oil in the laboratory, the above-mentioned ice crystals melted, which change probably gave lower suction force than would otherwise have been obtained. The remarkably relatively high suction force shown by these submersed plants, especially in summer, can possibly be partially explained by the fact, already known, that growing cells show a much higher suction force than others which are otherwise similar but are not growing. Thus, Ursprung and Blum found in the same growing root apex of *Vicia Faba* a suction force of 10 atm. in the elongating zone, while a few millimeters further back they found only 0.4 atm. Likewise, a geotropically curved radicle of *Vicia Faba* showed on its concave side a suction force of 0.5 atm., and on its convex side one of 8.2 atm. Again, in a bent pulvinus of *Tradescantia*, the pith of the concave side showed 2.7 atm., while that of the convex side showed 7.0 atm. (13, 26).

#### Laboratory Observations

I experimented with three young *Zea Mays* water cultures. Under similar conditions of light and atmospheric moisture (100 %), these plants were transferred from a room temperature of 15° C. to temperatures of 30°, 4°, and - 2° to + 9° C.

The upper halves of the primary leaves of the plants were measured on Feb. 4, 1924, at 5:00 P.M., and then were exposed to the given temperatures until Feb. 6 at 3:00 P.M. Then the lower halves of the same leaves were again measured. Before the experiment the average suction force of the upper halves of the leaves of the plants examined was 5.5 atm.

TABLE 28. *Zea Mays*

Variation of Suction Force in Comparison with Control (at 15° C.) for Plants Exposed to Temperature of

+ 30° C.	+ 4° C.	- 2° to + 9° C.
- 1.0 atm.	+ 0.6 atm.	+ 2.2 atm.

This comparison with the control plant at 15° C. shows that the suction force of the plant exposed to a higher air and culture-medium (water) temperature fell, while that of those exposed to lower temperatures rose.

In the experiment reported in table 28 the temperature of the air and soil (in this case distilled water) fluctuated as in nature.

It was essential to determine the influence of the temperature of the soil alone. Hence Hayoz made the following experiment: While the leaves of a number of *Zea Mays* plants were kept at laboratory temperature, the vessels containing their roots in water were exposed for a period of 12 hours to constant temperatures of - 12°, 0°, + 15°, and + 30° C.

After transferring the roots of one plant from water at 15° C. to that at 0° C., the suction force rose 4.6 %. A similar rise was noted after carrying roots of another plant from water at 15° to that at - 12° C. After transferring other roots from water at 15° to that at 30° C. no suction-force change was noted. From these experiments it is seen that the variation of soil and root temperature, alone, produces changes in suction force



similar to those which are caused by the variation of the temperature of the entire plant.

On the contrary, the lowering of the leaf temperature alone does not produce a rise similar to that which occurred when the temperature of the roots was also lowered. This was proved by the last-mentioned experimenter, who, for a period of 12 hours, transferred leaves from a temperature of  $18^{\circ}$  to one of  $-3^{\circ}$  C. The roots of one plant, however, were also transferred from water at  $18^{\circ}$  to that at  $-3^{\circ}$  C., while those of the other were transferred from water at  $18^{\circ}$  to that at  $+30^{\circ}$  C. In the former case, the suction force rose *ca.* 6 atm., in the second it remained constant.

Further experiments with *Bellis* were made in a slightly different manner. A leaf was cut lengthwise into halves, and each half was wrapped in tin foil. One half was kept at  $20^{\circ}$  C., the other was exposed for 13 hours to  $-1^{\circ}$  C. Thereupon, the suction force of the half which had been cooled was found to be 8.1 atm., and that of the control half leaf, 7.5 atm. Another similar experiment gave 8.1 atm. and 7.1 atm.

In contrast, the same plant which, in the open, had cooled to  $-1^{\circ}$  C. showed a rise in suction force to 14 atm. and higher. Here again we come to the conclusion that the suction-force increase of leaves in winter depends not so much upon the lowering of the temperature of the leaves as upon the increased difficulty experienced by the roots in taking up water, *i.e.*, it is a result of a disturbance in the water balance.

### Light and Heat Rays

#### *Field Observations*

In nature, it was hardly possible to study the effect produced by solar light- and heat-rays upon the suction force of plants. This was due to the fact that other factors exerted at the same time a much greater influence than that produced by the factor to be studied. This will be clearly demonstrated in what follows.

#### *Laboratory Observations*

A large Osram lamp, and a Lilliput arc lamp with lens, served as sources of light. The heat rays were produced by a hot plate.

According to thermo-electric measurements, the radiated energy, at the distance at which the plants were examined, was found to be:

Osram lamp. Total radiation.....	0.001 cal.	$\frac{\text{cm.}^2}{\text{sec.}}$
Osram lamp. Light radiation. (A plane parallel cuvette, 22 cm. broad, filled with running water, inserted between plant and source of light.) .	0.0001 cal.	$\frac{\text{cm.}^2}{\text{sec.}}$
Lilliput arc lamp with lens. Total radiation.....	0.01 cal.	$\frac{\text{cm.}^2}{\text{sec.}}$
Lilliput arc lamp with lens. Light radiation.....	0.001 cal.	$\frac{\text{cm.}^2}{\text{sec.}}$
Solar radiation, May 19, 1924, 3.00 P.M. Total radiation.....	0.005 cal.	$\frac{\text{cm.}^2}{\text{sec.}}$

Three young water cultures of *Zea Mays* were exposed for a period of 48 hours, at 1 m., 3 m., and 5 m., in front of the above-mentioned Osram lamp. Between it and the plants, a plane parallel cuvette 22 cm. broad, filled with running water, was inserted. As control I used a fourth plant, placed in the dark, whose temperature and air humidity were kept the same as those of the other three plants.

As will be seen in table 29, no striking variation in suction force was noticeable.

TABLE 29

Variation in Suction Force, as Compared with Control Plant (in Dark), of *Zea Mays* Leaves Exposed to a Radiated Light Energy of

0.0001 cal. $\frac{\text{cm.}^2}{\text{sec.}}$	0.000011 cal. $\frac{\text{cm.}^2}{\text{sec.}}$	0.000004 cal. $\frac{\text{cm.}^2}{\text{sec.}}$
+ 0.6 atm.	+ 0.8 atm.	+ 0.3 atm.

Further experiments by Hayoz were performed as follows: Young potted *Zea Mays* plants were so placed behind a light and heat screen that only the upper halves of the given leaves were exposed to the heat and light radiations, while the lower halves served as controls. The normal relation between these two leaf sections had been determined in advance (see table 19).

TABLE 30. *Zea Mays*

Radiated Energy Produced by	Plant Exposed during	Variation in Suction Force
Osram lamp, total radiation . . . . .	16 hrs.	Not determinable
Arc lamp, total radiation . . . . .	2 hrs.	Not determinable
Arc lamp, total radiation . . . . .	4 hrs.	Not determinable
Arc lamp, total radiation . . . . .	6 hrs.	Not determinable
Arc lamp, total radiation . . . . .	6½ hrs.	Not determinable
Hot plate, heat radiation. (An ordinary thermometer hung next to the leaf examined showed 50° C.) . . . . .	3½ hrs.	Not determinable

Hayoz exposed the corollas of several potted *Anemone hepatica* plants to various heat and light radiations. The suction forces of these corollas were determined before and after each experiment. The results are given in table 31.

TABLE 31. *Anemone hepatica*

Radiated Energy Produced by	Plant Exposed during	Suction Force before Exposure	Suction Force after Exposure	Suction Force Variation
Arc lamp, total radiation . . . . .	5 hrs.	16.0 atm.	17.8 atm.	+1.8 atm.
Arc lamp, light radiations concentrated on flower by means of lens (a plane parallel cuvette 22 cm. broad, filled with running water, inserted between plant and source of light) . . . . .	4½ hrs.	14.3 atm.	14.3 atm.	+0.0 atm.
Hot plate, heat radiation (thermometer with blackened bulb next to flower showed 35° C.) . . . . .	5 hrs. 7 hrs.	14.3 atm. 14.3 atm.	16.0 atm. 16.0 atm.	+1.7 atm. +1.7 atm.

## Wind

### *Field Observations*

In the month of February, 1924, the ray florets of *Tussilago Farfara* were measured. The plant grew on a hillside and was well exposed to the wind. The soil was thoroughly frozen. On Feb. 25, 1924, at 8 A.M., a time when there was no wind, the suction force measured 18 atm. During the following night a brisk wind blew, and the next day, Feb. 26, at 8 A.M., the suction force had risen to 23 atm. That this rise was, in all probability, due to the wind can be seen from parallel measurements made on the perianth of *Helleborus foetidus*. Simultaneously with the first *Tussilago* measurement, *Helleborus* showed a suction force of 66 atm.; when the suction force of *Tussilago* was determined the second time, *Helleborus* registered only 30 atm. The night before the second suction-force determination, *Helleborus* was covered with snow, and therefore was protected against the wind; *Tussilago*, because of its location and exposure upon a steep hillside, was free from snow, and hence the wind could play freely upon it. *Helleborus* dropped 36 atm., *Tussilago* rose 5. Since the temperature rose, I concluded that this difference was due to the wind or to increased transpiration.

### *Laboratory Observations*

The wind with which the following experiments were performed was produced by two electric fans. The smaller was capable of producing a breeze with a maximum velocity of 7-8 m. per second, while the other was capable of a wind velocity of 25 m. per second.<sup>7</sup>

The wind did not begin to produce any change upon the suction force of distilled-water cultures of *Zea Mays* until sufficient osmotically active substances were added to the culture medium (distilled water). As in all preceding laboratory experiments with *Zea Mays*, I determined the suction force on the same leaf before and after each experiment. In order to avoid errors caused by diurnal suction force variations, a control plant, not exposed to the wind, was kept in the same room where the other *Zea Mays* cultures were experimented upon.

In experiments made on potted *Chrysanthemum frutescens*, no effect of the wind upon the suction force of the petals and leaves was noticeable until the originally water-saturated soil became drier. The same potted plant was thoroughly watered the night before it was exposed to the wind. Immediately preceding the experiment, pot and soil were covered with tin foil. Hence, the water given off was transpired directly by the plant. In all the following experiments the petals were measured before and after each exposure to the wind, and, where possible, were taken from the same

<sup>7</sup> I wish here to express my thanks to Prof. P. Joye for his kindness in allowing me to use the large electric fan for these experiments.

TABLE 32. *Effect of Wind on Suction Force*

Name of Plant	Organ Examined	Location	Date, 1924	Rel. Air Humidity, %	Air Temp.	Wind † Velocity in m./sec.	Suction Force in Atm.	Suction Force Variation in Atm.
<i>Tussilago Farfara</i> . . . .	Ray floret	Edge of wood, steep hillside	2/25, 8 A.M.	97	-2°	0.0	18.0	
<i>Helleborus foetidus</i> . . . .	Perianth	Edge of wood	2/25, 8 A.M.	97	-2°	0.0	66.0	
† <i>Tussilago Farfara</i> . . . .	Ray floret	Edge of wood, steep hillside	2/26, 8 A.M.	96	-1°	10.0	23.0	+ 5.0
* <i>Helleborus foetidus</i> . . . .	Perianth	Edge of wood	2/26, 8 A.M.	96	-1½°	10.0	30.0	- 30.0

\* Covered with snow.

† Not covered with snow.

‡ Taken from tables of Freiburg Meteorological Station.

TABLE 33. *Zea Mays*

Cultivated in	Wind Velocity	Length of Time Exposed to Wind	Average Suction Force Found in Plants of Each Group at Start of Experiment	Variation in Suction Force in Comparison with Control Plant
Distilled water.....	5 m./sec....	8 hrs.	5.8 atm.	- 0.7 atm.
Distilled water.....	10 m./sec....	8 hrs.	5.8 atm.	- 0.5 atm.
Distilled water.....	15 m./sec....	8 hrs.	5.8 atm.	- 0.5 atm.
0.10 M. cane sugar.....	5 m./sec....	8 hrs.	7.4 atm.	- 1.3 atm.
0.10 M. cane sugar.....	10 m./sec....	8 hrs.	7.4 atm.	- 0.4 atm.
0.10 M. cane sugar.....	15 m./sec....	8 hrs.	7.4 atm.	- 1.7 atm.
0.20 M. cane sugar.....	5 m./sec....	8 hrs.	7.7 atm.	+ 4.3 atm.
0.20 M. cane sugar.....	15 m./sec....	8 hrs.	7.7 atm.	+ 4.1 atm.

capitulum (see page 439). Comparing the suction force of the plant placed 8 to 9 hours in winds of 3 m./sec., 4-5 m./sec., and 6 m./sec. respectively, with that of a control plant kept near the former plants, but not exposed to the wind, I found no notable rise in suction force in the former (table 34).

TABLE 34. *Chrysanthemum frutescens*

Velocity of Wind	Length of Time Exposed to Wind	Suction Force of Plant at Start (Petals)	Variation in Suction Force in Comparison with Control Plant
3 m./sec.....	8 hrs.	5.8 atm.	0.0 atm.
4-5 m./sec.....	4½ hrs.	5.3 atm.	+ 0.3 atm.
4-5 m./sec.....	9 hrs.	5.3 atm.	+ 1.1 atm.
6 m./sec.....	4 hrs.	4.8 atm.	- .9 atm.
6 m./sec.....	9 hrs.	4.8 atm.	- .3 atm.

As this experiment shows, a day's exposure to the wind produced no marked change in suction force; hence, in the experiment following (table 35) I placed the same plant daily, for 8 to 9 hours, in a wind of 6 m./sec. until it was entirely wilted. At the start of the experiment the soil of the plant and that of the control had been thoroughly soaked, and the pot and soil of each had been covered with tin foil. As will be noted from table 35, the suction force and transpiration of both plants were measured twice daily, morning and evening. At the commencement and termination of the experiment I determined the water content of a portion of the soil of each pot. Before studying the results, it is necessary to mention that both plants were about equal in size, and that the control was in a much more sandy soil than the plant exposed to the wind.

The last column of table 35 shows that the suction force of the plant exposed to the wind, as compared with that of the control, began to rise only when its soil became drier. That it did not rise sooner was probably

TABLE 35. *Chrysanthemum frutescens*

Date, 1923	No. Hrs. Exposed to Wind of 6 m./sec.	Rel. Air Humid- ity, %	Temp., ° C.	Transpiration in Grams				Water Content of Soil in Percentage of		Suction Force in Atm. of		Variation in Suction Force of Plant Exposed to Wind in Comparison with Control
				of Plant before Electric Fan		of Control		Plant before Electric Fan	Control	Plant before Electric Fan	Control	
				Total	Per Hr.	Total	Per Hr.					
10/24, 7 A.M.	Start	65	18					50.0	50.0	4.8	2.9	-0.9 atm. -0.3 atm.
11 A.M.	4 hrs.	68	18	32.8	3.6	44.7	5.0			5.6	4.6	
4 P.M.	9 hrs.	70	19	27.0	1.8	52.1	3.5			5.4	3.8	
Night 15 hrs.												
10/25, 7 A.M.	9 hrs.	70	20	60.5	6.7	50.0	5.6			6.2	5.7	-0.5 atm.
4 P.M.	18 hrs.	65	22	55.6	3.5	82.2	5.1			5.6	5.6	
Night 16 hrs.												
10/26, 8 A.M.	18 hrs.	69	21	59.5	6.6	50.5	5.6			5.0	6.0	+0.1 atm.
5 P.M.	27 hrs.	70	22	63.5	4.2	82.0	5.5			6.4	7.3	
Night 15 hrs.												
10/27, 8 A.M.	27 hrs.	55	21	44.0	5.2	25.5	3.0			6.8	8.8	-1.9 atm.
4:30 P.M.	35½ hrs.	72	18	60.8	3.8	30.3	1.9			7.1	11.0	
Night 16 hrs.												
10/28, 8:30 A.M.	35½ hrs.	65	19	16.2	2.0	15.0	1.9			9.8	11.1	+1.5 atm.
4:30 P.M.	43½ hrs.	65	20	22.0	1.4	24.0	1.5			11.9	11.7	
Night 16 hrs.												
10/29, 8:30 A.M.	43½ hrs.	67	21	14.5	1.8	11.5	1.4			11.5	11.7	+4.7 atm.
4:30 P.M.	51½ hrs.	70	19½	15.8	1.0	16.5	1.0			16.0	11.5	
Night 15½ hrs.												
10/30, 8:15 A.M.	51½ hrs.	70	18							15.5	12.1	+3.5 atm.
4:15 P.M.	59½ hrs.	67	19	10.7	1.4	8.0	1.0	15.0	13.3	19.6	12.7	
										Dead	Wilted	
												Sum of variations ... +6.2 atm.

because the soil of the control plant was more sandy than that of the one exposed to the wind. Sand, as is well known, exercises less tension on water than humus; hence roots in saturated sand can much more readily take up water. The result was that the suction force of the control, which was at first the lower, soon rose more rapidly; consequently, the plant exposed to the wind could not overtake it until the fifth day. Summarizing the experiment, I found that the sum of the variations, measured only during the periods of exposure, showed that the suction force of the plant exposed to the wind had risen a total of 6.2 atm. more than had the suction force of the other plant during the same periods.

The transpiration of the plants also corresponds with the facts above noted. On the first day the control gave off more water than did the plant exposed to the wind: 32.8 g. compared with 44.7 g. Again during the first three nights, when both plants were exposed to like meteorological conditions, the control transpired more than the other. On the morning of the fourth day, one fourth of the flowers of the control were drooping while practically all flowers of the other plant were normal. On the fifth day, three fourths of the flowers of the control plant, and only one half those of the other plant, were drooping. On the sixth and final day, practically all the flowers of both plants drooped.

Another interesting fact is that at the end of the experiment, the plant in the wind, which grew in good humus, contained a greater percentage of water than the control plant, although its suction force was higher and although the water content of the soil of both plants at the start of the experiment was 50 %. This shows, as stated above, that humus holds its water with greater tenacity than sand.

That laboratory plants manifest a diurnal periodicity can be seen from table 35 and the following tables.

Table 36 shows the results of an experiment on another potted *Chrysanthemum frutescens* with water-saturated soil. The pot, but not soil, covered with tin foil was placed before a wind with a velocity of 15 m./sec. The suction force, as compared with the control, began to rise on the first day. After 4 days the sum of the variations was 10 atm.; the next morning all the leaves and flowers were wilted.

The transpiration column shows some interesting data. At night both plants gave off about the same quantity of water. During the day the plant exposed to the wind gave off 2 to 6 times as much as the control. The first day they transpired 105.3 g. and 17.5 g., the last 40.8 g. and 16.8 g. respectively. The water content of the soil of the two plants decreased correspondingly.

From twenty measurements made twice daily, the mean width of the stomatal apertures of the leaves shows that, in general, the apertures of the exposed plant were narrower than those of the control. Similarly, the infiltration was, as a rule, less for the exposed plant than for the control.

TABLE 36.\* *Chrysanthemum frutescens*

Date, 1923	No. Hrs. Exposed to Wind of 15 m./sec.	Rel. Air Humidity, %	Temp., ° C.	Transpiration in Grams				Water Contents of Soil in Percentage of	Width of Stomatic Pores in $\mu$							
				of Plant Exposed to Wind		of Control			of Plant Exposed to Wind			of Control Plant				
				Total	Per Hr.	Total	Per Hr.		Plant Exposed to Wind	Control	Ave.	Max.	Min.	Ave.	Max.	Min.
11/16, 9:30 A.M.	Start	70	16	105.3	15.0	17.5	2.5	33.6	46.4	4.0	9.0	5.1	9.0	0.8		
4:30 P.M.	7 hrs.	78		33.2	2.0	25.0	1.5	16.9	41.6	1.5	3.0	4.3	12.0	1.5		
11/17, 8:30 A.M.	16½ hrs.	65	16													
11/17, 4:30 P.M.	7 hrs.	65		96.5	12.1	24.2	3.0	12.0	38.1	2.0	5.0	2.5	6.8	0.8		
11/18, 8:30 A.M.	16 hrs.	65	15	22.3	1.4	18.8	1.2			2.4	12.0	0.0	4.5	0.8		
11/18, 4:30 P.M.	15 hrs.	65	15	37.2	4.7	17.2	2.2	9.2	31.7	2.3	3.8	3.9	9.0	0.8		
11/19, 8:30 A.M.	23 hrs.	62	15	13.1	0.8	12.6	0.8			1.1	2.3	2.1	3.8	0.0		
11/19, 4:30 P.M.	16 hrs.	62	14													
	23 hrs.	60		40.8	5.1	16.8	2.1	4.1	28.0	0.7	2.3	1.5	4.5	0.0		
	31 hrs.										4.5	1.6	2.3	0.0		

Infiltration into Stomatal Apertures of								Suction Force in Atm. of		Variation in Suction Force of Plant Exposed to Wind in Comparison with Control
Plant Exposed to Wind				Control Plant				Plant Exposed to Wind	Control	
Petroleum Ether	Ethyl Ether	Xylol	Ethyl Alcohol	Petroleum Ether	Ethyl Ether	Xylol	Ethyl Alcohol			
Very strong Nil	Strong Nil	Weak Nil	Nil Nil	Very strong Very strong	Strong Strong	Weak Weak	Nil Nil			
Very strong Weak	Strong Weak	Very strong Nil	Nil Nil	Very strong Very strong	Strong Strong	Nil Weak	Nil Nil			
Weak Very strong	? Weak	? ?	Nil Nil	Very strong Weak	Strong Strong	Strong Weak	Nil Nil			
Weak Weak	Very strong Very strong	? ?	Nil ?	Strong Very strong	Very strong Very strong	Weak Weak	Nil Nil			

+3.2 atm.	+0.9 atm.	+2.0 atm.	+3.9 atm.
5.8 4.4	5.0 5.0	5.9 4.4	4.0 3.3
7.2 10.4 Dead	7.8 5.9	7.2 10.4 Dead	3.3 Fresh

Sum of Variations . . . +10.0 atm.

\*The lower half of this table should follow to the right of the upper half. It is thus arranged for lack of space.



In explanation of the peculiar behavior of the suction force of the control during the experiment, I can suggest only that perhaps the soil during the first few days was so saturated with water that it lacked oxygen. This want of oxygen might have produced the higher suction force at the beginning.

In tables 35 and 36 are given the results of two experiments, during which I had occasion to observe the injurious effects produced by wind upon plant tissues. Leaves, sometimes as early as the first day, began to be discolored at their tips. The discolored spots grew in size and in number as time proceeded. At the end of the experiment, most of the leaves of the plant exposed to a wind of 15 m./sec. were half dried. When the wind was very strong, the branches with their leaves pointed in the direction of the wind. As a rule, the spots started at the outer edges of the leaves and grew inward. Only when they resulted from mechanical injuries did such spots begin at the interior of the leaves. As to the petals, when the wind was very strong they became brown before they had a chance to wilt; when the wind was weaker it frequently happened that the petals wilted first, and later became brown and shriveled. I noticed in wind experiments upon plants, as had Hansen (6), that spots appeared on leaves which otherwise were fully turgescient. The granular plasma and brown vessels, observed in leaves by Hansen, I also saw very clearly in petals (10, 12).<sup>8</sup>

#### Oxygen Content of the Soil

That the lack of oxygen in the soil or culture liquid causes the suction force to rise has already been pointed out by Ursprung and Blum (24). They transferred *Vicia Faba* seedlings from sawdust, in one case, to ordinary tap water containing air, and in a second case, to water covered with oil, after the air had been driven away by boiling. After a few days, the suction force fell from 1.1 atm. to 0.0 atm. in the plant growing in the water containing air; in the oxygen-free water it remained constant until the plant began to wilt.

#### Field Observations

In a number of cases, plants growing in soil completely saturated with water showed a higher suction force than the same species in less moist soil. Most striking was the case when, twice in the same day, I determined the suction force of a *Solanum nigrum* growing in running water in front of a molasse wall. Parallel measurements were made on another plant of the same species growing in less moist but good soil, 50 feet away. In the former plant I found suction forces of 6.5 atm. and 8.0 atm., in the latter of 5.5 atm. and 6.0 atm. respectively. Even though all other exterior factors were probably not identical for both plants, nevertheless it seemed that the difference might possibly have been due chiefly to the different oxygen content of the soil (10). (See *Solanum nigrum*, table 20.)

<sup>8</sup> The work of Bernbeck (Flora N.F. 17: 292. 1924) came to my attention only when this paper was in the course of printing, and could not be taken into consideration.

*Laboratory Observations*

Experiments with water cultures of *Zea Mays* were recently made by Hayoz. The roots of the plants had been kept in ordinary tap water for a number of days. In the meantime the air in other tap water had been driven off by boiling. After the water had fallen to room temperature, the roots of a few of the above-mentioned plants were placed in it. Then the water was covered with a layer of oil to keep out oxygen. Control plants were kept in the first-mentioned tap water. As tables 37 and 38 show, the suction force of plants in water, freed from oxygen, rose.

TABLE 37. *Zea Mays*

Time during which Plant was in Tap Water Freed from Oxygen	Suction Force of Leaves	
	Tap Water Freed from Oxygen	Control Plant in Ordinary Tap Water
0 days (original value).....	5.3 atm.	} .....6.7 atm.
1 day.....	6.7 atm.	
2 days.....	9.6 atm.	

TABLE 38. *Zea Mays*

Time during which Plant was in Tap Water Freed from Oxygen	Suction Force of Leaves	
	Tap Water Freed from Oxygen	Control Plant in Ordinary Tap Water
0 days (original value).....	5.3 atm.	
1 day.....	5.3 atm.	4.0 atm.
2 days.....	9.6 atm.	5.3 atm.
3 days.....	9.0 atm.	5.9 atm.

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## A STUDY OF SUCTION FORCE BY THE SIMPLIFIED METHOD II. PERIODIC VARIATIONS AND THE INFLUENCE OF HABITAT

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### PERIODIC VARIATIONS IN SUCTION FORCE AND THEIR EXPLANATION

#### The Diurnal Periodicity

I did not propose to myself to make a study of periodic variations of suction force. That was being done by T. Kandija. However, among my observations there is an accumulation of data which may also throw light upon this question. Thus, as will be seen from table 21, on Sept. 11, 1923, at 1:30 P.M., *Trifolium pratense* had shown a suction force of  $1\frac{1}{2}$  atm. higher than it did at 8:00 A.M. on the same day and on the following day. Again, the corolla of *Epilobium angustifolium* registered a suction force of 6.0 atm. at 8:00 A.M., July 10, 1923, while the same organ of the same plant at 4:15 P.M. registered one of 8.0 atm. Much greater fluctuations, amounting to over 10 atm. daily, were found by Kandija in the ligulate flowers of *Bellis perennis*.

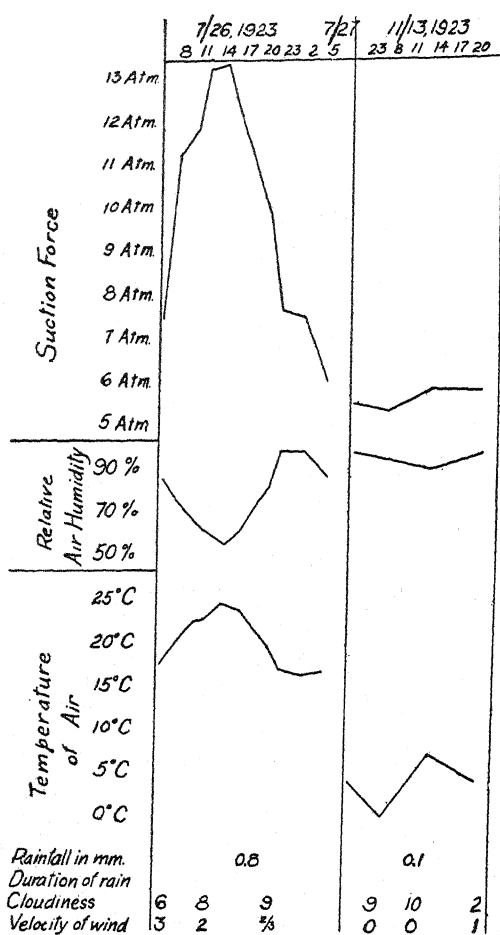
On normal days in summer, when there are no extraordinary atmospheric disturbances, the suction-force curve rises from the morning until noon, then falls until the next morning. Thus, on July 26, 1923, the suction force of the ligulate flowers of *Bellis* (text fig. 2) increased from *ca.* 7.5 atm. at 6:00 A.M. to *ca.*  $13\frac{1}{2}$  atm. at about 2:00 P.M.; then it fell to a minimum of *ca.* 6 atm. on the next day at 5:00 A.M.; from this time on it rose once more.

In seeking the effective causes of these suction-force variations, it is important to know that the transpiration curve follows a similar course (14). This fact points to relations existing between the water balance and suction force. A proof of these relations seems to lie in the comparison between the suction-force curve and the swelling curve (16, 2). With a decrease in the swelling of an organ the suction force rises, and *vice versa*.

Ursprung and Blum (16) observed that the osmotic value shows daily variations similar to those of suction force, the maximum being at noon. Since the diurnal curves of swelling and of osmotic value proceed in opposite directions, they must mutually support each other. Hence the suction-

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force variations, which depend on changes in volume, are further increased by the fluctuations in osmotic value. The chief rôle seems to be played by fluctuations in volume, for the osmotic value, according to our present knowledge, varies much less than the suction force. The latter fluctuates daily in the corolla of *Bellis* over a range of 10 atm., a range which will probably even be exceeded if the proper plant is found.



TEXT FIG. 2. Diurnal periodicity of ligulate florets of *Bellis*.

Among the factors that show a daily similar or contrary curve to that of suction force is, above all, relative air humidity. As has been seen, suction force rises with decreasing atmospheric humidity (text fig. 2, July 26). From this fact it follows that the suction-force curve depends, at least qualitatively, on the moisture variations. Both cell volume and osmotic value, the components of suction force, react, as far as known, in

such a way that decrease in air moisture is always attended by increase in suction force.

Air and soil temperature (5)<sup>1</sup> also show diurnal periodic variations (text fig. 2, July 26); but since, when air and soil temperatures increase, the suction force, *ceteris paribus*, falls, these temperature variations can not give the explanation that we are seeking.

Again, the solar radiation which falls upon plant and soil shows a marked diurnal periodicity, having a maximum at noon (5). Nevertheless, as far as the suction-force curve is concerned, this factor need not be considered; for, first, the effect of light and heat rays upon suction force is extremely slight, and second, the curve of suction-force variation even on perfectly sunless days is normal, provided that the curve of atmospheric humidity varies correspondingly.

The very form of the atmospheric pressure curve (5), with its two maxima, at 10:00 A.M. and 10:00 P.M., clearly indicates that it is not related to the suction-force curve. The same statement applies to the atmospheric-ionization (conductivity) curve, whose maxima and minima likewise lie differently (5, 9, 11).<sup>2</sup>

Rain and wind are not to be considered, for they show no corresponding periodicity.

Among the hitherto-mentioned external factors, atmospheric moisture, at least according to our present knowledge, *primarily* causes the regular diurnal periodicity of suction force. This will be seen by a close examination of the curves of text fig. 2, which on July 26, 1923, were practically similar but contrary in direction. Again, on Nov. 13, 1923 (text fig. 2), the air moisture remained practically constant, as did also the suction force.

Comparing the suction-force fluctuation of 6 atm. with the corresponding air-moisture variation of 40 percent (text fig. 2, July 26, 1923), or comparing the same plant of *Bellis perennis* that was used in the laboratory experiment, in which an air-moisture variation of 40 percent corresponded with a suction-force variation of 6.2 atm. or even 7.3 atm. (table 25), we see that the daily suction-force variations can be traced back, both qualitatively and quantitatively, to corresponding air-moisture variations.<sup>3</sup> As already

<sup>1</sup> At the very spot where Kandija examined *Bellis*, he made the following readings:

Hour	6 P.M.	8 P.M.	10 P.M.	4 A.M.	6 A.M.	8 A.M.	10 A.M.	12 A.M.	2 P.M.	4 P.M.	6 P.M.	8 P.M.
Air temp.....	20.5	16.4	14.0	10.5	10.5	13.5	17.8	19.3	21.0	21.3	20.0	16.2
Soil temp.												
(3 cm. deep)...	20.8	18.5	16.0	12.0	13.0	13.8	16.5	18.0	18.7	18.8	18.5	17.4
Soil temp.												
(10 cm. deep)...	20.8	19.3	18.2	15.4	14.7	14.6	15.1	16.2	17.0	17.5	17.6	17.4

<sup>2</sup> According to oral information received from Prof. A. Gockel, the curve in summer, for Freibourg, Switzerland, has during the daytime a minimum between 11:00 and 12:00 A.M. and a maximum between 4:00 and 5:00 P.M.

<sup>3</sup> It would have been very instructive to include the curve of the saturation deficit in text figure 2, for this curve runs in a path more or less nearly parallel to the path of the

TABLE 39. *Diurnal Periodicity of Indoor Cultures*

Name of Plant	Culture Medium	Date	Rel. Air Humidity, %	Air Temp., ° C.	Weather	Suction Force in Atm.
<i>*Zea Mays</i> .....	Distilled water	1/11/24, 8:30 A.M.	62	13	Cloudy	5.8
	Distilled water	1/11/24, 4:00 P.M.	54	19	Bright	7.3
	0.10 M. Cane sugar solution	1/15/24, 8:00 A.M.	54	13	Bright	4.2
	0.10 M. Cane sugar solution	1/15/24, 4:00 P.M.	50	17	Bright	5.4
<i>Chrysanthemum frutescens</i> .....	0.20 M. Cane sugar solution	1/18/24, 8:00 A.M.	57	14	Cloudy	6.9
	0.20 M. Cane sugar solution	1/18/24, 4:00 P.M.	50	19	Cloudy	7.1
	Soil (potted plant)	10/19/23, 2:00 P.M.	60	18	Bright	7.6
	Soil (potted plant)	10/20/23, 8:00 A.M.	69	16	Cloudy	6.7
	Soil (potted plant)	10/19/23, 5:00 P.M.	63	18	Cloudy	6.3
	Soil (potted plant)	10/20/23, 8:00 A.M.	69	16	Cloudy	5.9
	Soil (potted plant)	10/23/23, 7:00 A.M.	65	20	Bright	5.3
	Soil (potted plant)	10/23/23, 11:30 A.M.	65	22	Cloudy	5.3

\* Attention should be called to the fact that the suction-force variation in the water cultures of *Zea Mays* noted above was abnormally high.

noted, similar variations in atmospheric moisture accompanied by different variations in soil moisture produce different suction-force fluctuations.

To supplement the observations made in the field we now note facts which came to our observation in the laboratory. Here, too, there were diurnal suction-force variations which, in all probability, were also caused by fluctuations in atmospheric moisture. (See table 39.)

### Annual Periodicity

The researches made by me relative to annual periodicity cover a period of a few weeks only. I experimented upon the standard of the corolla of *Trifolium pratense* and upon the lower foliar epidermis over the base of the midvein in the middle leaflet of *Helleborus foetidus*.

In the standard of the corolla of *Trifolium pratense* (text fig. 3), the suction force fell from August 31 to September 5. This drop was evidently due to the rain which had fallen on August 31. From September 6 to September 10 it rose, probably because of the absence of precipitation during this period. The sudden fall from September 15, and the continued low suction-force values to the end of the month, correspond with frequent precipitations. During the period of dryness toward the beginning of October, the suction force rose slowly again. The rain on October 2 and following days caused it to fall once more.

It should not cause surprise that every small fluctuation of the suction-force curve can not be explained, for all measurements could not be made upon one and the same plant. Hence allowance must be made for the often relatively large individual fluctuations between plants.

The suction-force curve of *Trifolium* shows that the annual periodicity depends primarily upon the quantity and the distribution of precipitation. Air-moisture fluctuations, on the contrary, exerted only a secondary influence on the curve.

### THE INFLUENCE OF HABITAT UPON SUCTION FORCE

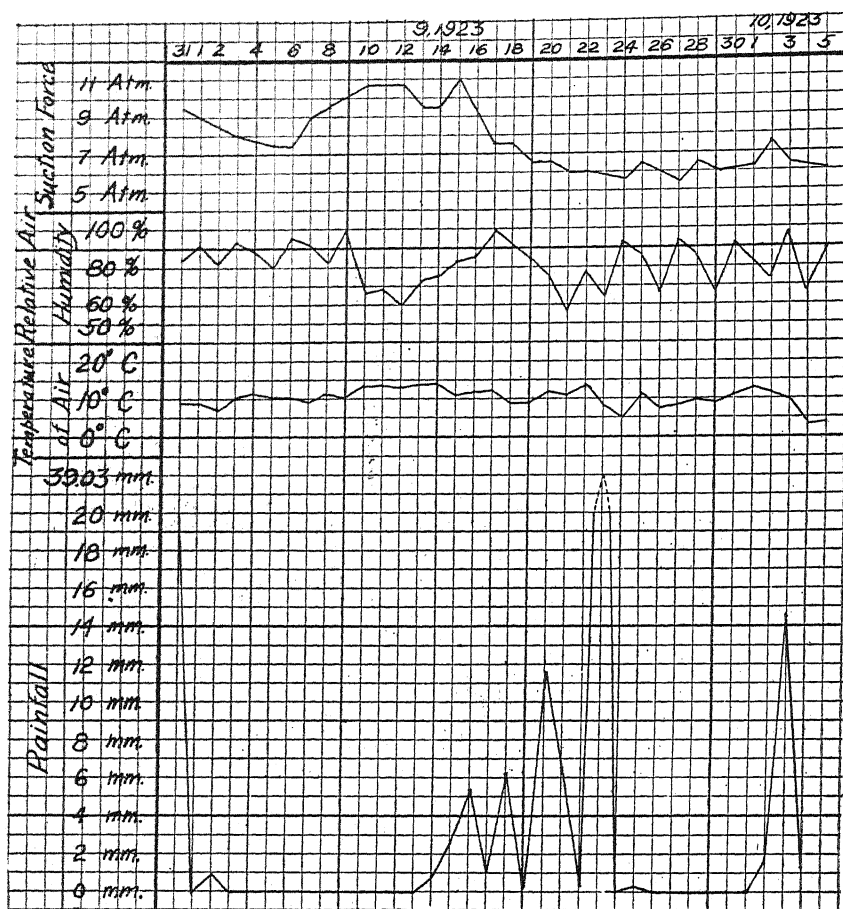
Judging from the high osmotic values that Fitting found in desert plants, we should be inclined to conclude that plants growing in relatively dry places show a relatively high suction force. This conclusion may be curve of suction force, and the evaporation is proportional to the saturation deficit. Nevertheless, I preferred to give only such meteorological values, in connection with suction force, as can be read directly. Thus space would not permit the inclusion of the graph for the saturation deficit, which can, however, be readily calculated at any time from the relative humidity and the temperature.

By way of illustration, I give the figures for July 26, 1923, in which those for the saturation deficit for each hour appear directly below those of the corresponding suction force.

	A.M.					P.M.		
	5:45	7:30	10:00	12:00	2:30	4:30	8:30	10:30
Suction force.....	7.7	11.2	11.9	13.3	13.5	12.2	9.9	7.8
Saturation deficit.....	2.2	3.0	7.0	8.0	9.7	8.1	2.6	0.0



tested by comparing a wood flora with that of a dry wall or pond. It should be remembered, however, first, that different species may show different suction forces even though growing in the same locality; second, that the same species in different habitats may have differences in structure,



TEXT FIG. 3. Periodicity of corolla of *Trifolium pratense*.

such as dissimilarities in the form of the root system or inequalities in the area of their absorbing and transpiring surfaces; and third, that even the same plant in the same location shows a diurnal and an annual periodicity in suction force.

From these considerations it will be seen that we should measure the suction force under absolutely identical conditions only if the same plant could be measured at the same time in different localities. This being out of the question, the best method under the circumstances appeared

to be to measure as many different species in as many different habitats as possible. From the material thus acquired I present:

1. Simultaneous measurements made upon the corollas of the same species in different habitats.
2. Aggregate measurements made upon the corollas of the same species in different habitats.
3. The mean suction force of the corollas of all plants of the same habitat.

The results obtained in group 1 are naturally the most reliable, but also the smallest in number; for it becomes more difficult to fulfill the requirement when the plants to be measured are far apart.

Group 2 is represented by a larger number of measurements. Because of periodic variations, these measurements made at different times would be strictly comparable only if by some method they could be reduced to the same time. At present it is not possible to do this accurately, since we have the fairly complete annual suction-force curve only of *Bellis perennis*. This had been worked out by Kandija.

Under these limitations we proceeded thus: for every suction-force value of group 2 the corresponding value of *Bellis perennis* (i.e., the simultaneously measured suction force of *Bellis*) was referred to and a comparison was made (14). In illustration, I here present a few measurements of *Taraxacum officinale*, one of a plant found in a meadow, and nine of plants found in a bog. The suction force of the *Taraxacum* plant in the meadow was 3.5 atm. (59.8 percent less than the corresponding value for *Bellis*, which was 8.7 atm.); the nine measurements of *Taraxacum* plants in the bog gave a mean of 8.8 atm. (1.1 percent less than the mean of the corresponding nine *Bellis* values, which was 8.9 atm.).

The comparisons in groups 1 and 2 can be made only for plants that occur in both localities; hence these comparisons are limited to a few species and exclude those which have characteristic habitats. The gap caused by this exclusion was filled out by the observations of group 3, involving also a comparison with corresponding *Bellis* values.

#### Simultaneous Measurements Made upon the Corollas of the Same Species in Different Habitats

Examining the separate pairs shown in table 40, we see that in general the plant with the relatively drier habitat has the higher suction force. Several exceptions deserve special consideration.

(a) It is striking that the suction force of *Solanum nigrum* growing in running water is higher than that of the same species growing along the roadside. Two factors may have been responsible for this. First, the plant, with roots and the base of its stalk in running water, grew before a molasse cliff exposed to the sun from morning until evening, the leaves and flowers thus being subjected to both direct and reflected rays of the

sun. Second, a lack of oxygen in the soil may also have played a rôle in causing an increase in suction force.

TABLE 40. *Suction Force of the Corollas of the Same Species in Different Habitats*

Name of Plants	Date, 1923	Suction Force of Corolla in Atm.					
		Humid Forest and Hedge-row	Wall and Conglomerate Boulder	Road-side	Clearing and Edge of Wood	Root and Base of Stem in Running Water	River Sand
<i>Geranium Robertianum</i> .....	6/25, 2:00 P.M.	5.5	5.5				
	7/12, 8:30 A.M.	6.0	8.0				
	7/13, 2:30 P.M.	6.5	6.5				
	10/ 2, 1:30 P.M.	2.5	6.0				
<i>Chelidonium majus</i> .....	7/13, 2:30 P.M.	5.5	9.5				
<i>Trifolium repens</i> .....	9/ 6, 2:45 P.M.			9.5	7.5		
<i>Potentilla anserina</i> .....	9/ 7, 10:30 A.M.			12.0	6.5		
<i>Trifolium pratense</i> .....	9/14, 8:15 A.M.			9.0	9.0		
	9/11, 8:15 A.M.			9.5	10.5		
	9/11, 1:15 P.M.			11.0	12.5		
<i>Melilotus albus</i> .....	9/17, 8:15 A.M.				7.5	6.0	
<i>Solanum nigrum</i> .....	9/15, 2:45 P.M.			6.0			6.0
	9/28, 9:30 A.M.			5.5		6.5	
	9/28, 2:30 P.M.			6.0		8.0	

(b) That the *Trifolium pratense* found at the edge of a wood should show a higher suction force than the one growing along the wayside may be explained by the circumstance that the former grew on a slope where the soil could dry out relatively fast. The plant with the higher suction force behaved much like the *Bellis* with which comparisons are made. The *Bellis* grew on the edge of a raised garden bed, and because of this showed great suction-force fluctuations. Furthermore, the cause of the relatively low suction force of the *Trifolium* exposed on the wayside probably lay in the fact that it was covered with dew while the other plant was not.

(c) That *Geranium Robertianum* frequently showed the same suction force in the drier habitat as in the moister one results from its peculiar and low capability of reacting against external factors. Comparing the behavior of these plants of *Geranium* with that of *Chelidonium majus*, both measured at the same time (July 13, 1923, 2:30 P.M.) and in the same habitats, we find that *Chelidonium* varies by fully 4 atm. (5.5 atm. as compared with 9.5 atm.) while *Geranium* remains constant (at 6.5 atm.).

(d) The extremely low suction-force value of the *Geranium* (2.5 atm.) measured on Oct. 2, 1923, at 1:30 P.M., evidently was due to the fact that the plant and its surrounding soil were covered with moisture blown upon them in a fine spray from a near-by waterfall. The other plant was measured at the same time upon a neighboring wall, but was not reached by the spray.

Table 40 may be briefly summarized by the following comparison, in which the suction forces of the plants with relatively moist habitats are compared with those of others with relatively dry habitats. The abnormal values of *Solanum* are not included.

Mean suction force of plants with relatively moist habitat, 7.0 atm.  
Mean suction force of plants with relatively dry habitat, 8.3 atm.

#### Aggregate Measurements Made upon the Corollas of the Same Species in Different Habitats

In table 41 are found all the measurements made upon the same species in different habitats. Since the measurements to be compared with one another were not always taken simultaneously, the corresponding values of the control *Bellis* are added in every case.

The data of table 41 are summarized in table 42. For every species the mean suction force of each habitat is calculated, the corresponding suction force for *Bellis* is given in brackets, and then, in percentage, is given the variation of the plants in question from the mean for the corresponding *Bellis*.

Practically all the results summarized in table 42 are easily accounted for, since the plants growing in the drier habitats have a higher suction force than those in the more moist. However, the comparison must be made with reference to the numbers expressed as percentages, for in them alone all important data have been considered.

Somewhat striking is the behavior of *Melilotus albus*. The important fact here is that the mean of the plants found along the railroad lies relatively low; no doubt this is due principally to the measurement (table 42) in which a *Melilotus* suction force of 6.5 atm. corresponds with a *Bellis* value of 21 atm. The astonishingly low value of *Melilotus albus* probably depends upon its deep and well developed root system, which during longer periods of drought succeeded in obtaining enough water to keep its suction force low; in the meantime, the *Bellis* roots had difficulty in absorbing enough water and the plant therefore developed a high suction force. The reason for the abnormal behavior of *Solanum nigrum* has already been given.

All the measurements of group 2 can again be summarized by taking the mean of the plants growing, first, in relatively moist, and second, in relatively dry, habitats. Then their variations in comparison with *Bellis* are calculated in percentages, and these values in turn are compared with one another.

Mean suction force of plants with relatively moist habitats, - 25 % variation  
Mean suction force of plants with relatively dry habitats, + 11.5% variation



TABLE 4I (continued)

Name of Plant	Suction Force of Corolla in Atm.													
	Humid Forest and Hedge-row	Bellis Control	Root and Base of Stem in Running Water	Bellis Control	Clearing and Edge of Wood	Bellis Control	Railroad and Wayside	Bellis Control	Wall Conglomerate and Molasse Boulder	Bellis Control	Molasse Cliff	Bellis Control	River Sand	Bellis Control
<i>Trifolium pratense</i> .....			6.0	8.5	11.0 11.0 10.5 10.5 9.5 8.0 7.5 11.0 7.5 9.5 6.5 9.0 9.5 8.0 7.5 7.5 9.0 5.5 7.0 6.0 5.0 5.5 9.0 13.0 10.5 9.5 9.5 11.0 7.5 7.5 6.5 6.0	15.5 18.5 14.0 21.5 18.0 22.5 11.0 15.0 11.0 9.0 8.0 6.0 10.5 5.0 7.0 6.0 5.5 7.0 5.5 7.0 5.0 9.0 13.0 9.5 8.5 9.0 5.0 6.0 6.0 5.0 7.5 6.0 5.5	9.5 11.0	9.0 13.0						

TABLE 41 (continued)

Name of Plant	Suction Force of Corolla in Atm.													
	Humid Forest and Hedge-row	Bellis Control	Root and Base of Stem in Running Water	Bellis Control	Clearing and Edge of Wood	Bellis Control	Railroad and Wayside	Bellis Control	Wall Conglomerate and Molasse Boulder	Bellis Control	Molasse Cliff	Bellis Control	River Sand	Bellis Control
<i>Trifolium pratense</i> .....					6.0 5.5 6.5 5.5 6.5 6.0 6.5 7.5 6.5 6.0	7.0 5.0 4.0 9.0 7.5 4.0 5.5 7.0 6.0 8.0								
<i>Erucastrum nasturtiifolium</i> .....							15.5	6.0					9.5	8.5
<i>Campanula rotundifolia</i> .....					8.0 9.0	11.0 10.5			15.5	8.0				
<i>Bellis perennis</i> .....			5.5	5.5			11.0	10.0	12.5	13.0			6.5	8.5
<i>Saponaria officinalis</i> .....			5.5	9.5			5.5 6.0	7.5 9.0						
<i>Solanum nigrum</i> .....			6.5 8.0	7.5 9.0										
<i>Trifolium repens</i> .....					7.5	10.0	9.5	10.0						

TABLE 42

<i>Hieracium murorum</i> , humid forest (2).....	7.3 atm. [8.3 atm.] <	15.0 atm. [15.5 atm.]	Conglomerate boulder (2)
	- 12.0%	- 3.2%	
<i>Geranium Robertianum</i> , humid forest (5).....	5.2 atm. [13.9 atm.] <	7.0 atm. [13.8 atm.]	Wall and conglomerate boulder (5)
	- 62.6%	- 49.3%	
<i>Epilobium montanum</i> , humid forest (1).....	8.0 atm. [8.0 atm.] <	7.8 atm. [7.4 atm.]	Edge of wood (4)
	0.0%	+ 5.4%	
<i>Chelidonium majus</i> , hedge (2)....	5.0 atm. [15.5 atm.] <	9.5 atm. [23.5 atm.]	Wall (1)
	- 67.7%	- 59.6%	
<i>Hypericum perforatum</i> , edge of wood (1).....	9.5 atm. [10.5 atm.] <	9.5 atm. [8.0 atm.]	Wayside (1)
	- 9.5%	+ 18.8%	
<i>Chrysanthemum Leucanthemum</i> , moist river sand (1).....	4.5 atm. [8.5 atm.] <	16.0 atm. [15.5 atm.]	Conglomerate boulder (2)
	- 47.1%	+ 3.2%	
<i>Potentilla anserina</i> , edge of wood (4).....	7.4 atm. [9.9 atm.] <	10.5 atm. [8.3 atm.]	Railroad (2)
	- 25.3%	+ 26.5%	
<i>Melilotus albus</i> , railroad (3).....	7.3 atm. [12.0 atm.] <	6.5 atm. [7.5 atm.]	River sand (1)
	- 39.2%	- 13.3%	
<i>Convolvulus sepium</i> , edge of wood (3).....	3.8 atm. [11.7 atm.] <	14.5 atm. [8.0 atm.]	Molasse cliff (1)
	- 67.5%	+ 81.3%	
<i>Erucastrum nasturtiifolium</i> , river sand (1).....	9.5 atm. [8.5 atm.] <	15.5 atm. [6.0 atm.]	Railroad (1)
	+ 11.8%	+ 158.3%	
<i>Campanula rotundifolia</i> , edge of wood (2).....	8.5 atm. [10.8 atm.] <	15.5 atm. [8.0 atm.]	Wall (1)
	- 21.3%	+ 93.8%	
<i>Bellis perennis</i> , stem and roots in running water (1).....	5.5 atm. [5.5 atm.] <	11.0 atm. [10.0 atm.]	Wayside (1)
	0.0%	+ 10.0%	
<i>Saponaria officinalis</i> , moist river sand (1).....	6.5 atm. [8.5 atm.] <	12.5 atm. [13.0 atm.]	Molasse boulder (1)
	- 23.5%	- 3.8%	
<i>Solanum nigrum</i> , wayside (2)....	5.8 atm. [8.3 atm.] <	6.7 atm. [8.7 atm.]	Stem and roots in run- ning water (3)
	- 30.1%	- 23.0%	
<i>Trifolium repens</i> , forest clearing (1).....	7.5 atm. [10.0 atm.] <	9.5 atm. [10.0 atm.]	Railroad (1)
	- 25 %	- 5 %	
<i>Trifolium pratense</i> , stem in running water (1).....	6.0 atm. [8.5 atm.] <	8.3 atm. [9.1 atm.]	Edge of wood (4)
	- 29.4%	- 8.8%	
<i>Trifolium pratense</i> , edge of wood (40).....	8.3 atm. [9.1 atm.] <	10.3 atm. [11.0 atm.]	Wayside (2)
	- 8.8%	- 6.4%	

**The Mean Suction Force of the Corollas of All Plants of the  
Same Habitat**

TABLE 43. *Fresh Water Pond (Plant, Excluding Flower, Submersed)*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Ranunculus flaccidus</i> .....	6/20, 8:30 A.M.	4.0	8.5	-52.9



TABLE 44. *Humid Forest and Hedgerow*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Aquilegia vulgaris</i> .....	6/ 4, 8:15 A.M.	6.5	7.0	- 7.1
		7.5*	7.0	+ 7.1
<i>Majanthemum bifolium</i> .....	6/ 5, 2:30 P.M.	6.0	7.0	-14.3
<i>Asperula odorata</i> .....	6/ 6, 8:30 A.M.	5.5	7.5	-26.7
	6/ 6, 5:15 P.M.	5.5	8.5	-35.3
<i>Ajuga reptans</i> .....	6/ 7, 8:30 A.M.	7.5	7.0	+ 7.1
<i>Hieracium murorum</i> .....	6/ 8, 5:30 P.M.	6.5	7.5	-13.3
	6/ 9, 11 A.M.	8.0	9.0	-11.1
<i>Phyteuma spicatum</i> .....	6/12, 2:30 P.M.	7.5	10.5	-28.6
<i>Geranium Robertianum</i> .....	6/12, 4 P.M.	4.5	9.5	-52.6
<i>Chelidonium majus</i> .....	6/13, 8:15 A.M.	4.5	7.5	-40.0
<i>Veronica Chamaedrys</i> .....	6/13, 4:45 P.M.	4.5	9.5	-52.7
<i>Fragaria vesca</i> .....	6/14, 8:15 A.M.	4.5	6.0	-25.0
<i>Veronica officinalis</i> .....	6/18, 2:15 P.M.	6.5	7.0	- 7.1
<i>Neottia Nidus-avis</i> .....	6/21, 10 A.M.	4.5*	11.0	-59.1
<i>Geranium Robertianum</i> .....	6/25, 2 P.M.	5.5	10.0	-45.0
<i>Epilobium montanum</i> .....	7/ 2, 3 P.M.	8.0	8.0	0.0
<i>Cephalanthera rubra</i> .....	7/ 4, 9 A.M.	9.0	6.0	+50.0
<i>Platanthera bifolia</i> .....	7/ 4, 9 A.M.	5.5	6.0	- 8.3
<i>Rosa arvensis</i> .....	7/ 5, 8:15 A.M.	10.5	6.0	+75.0
	7/ 7, 9:15 A.M.	9.5	6.0	+58.3
<i>Epilobium angustifolium</i> .....	7/10, 8:15 A.M.	6.0	13.5	-55.6
	7/10, 4:15 P.M.	8.0	17.5	-54.3
<i>Geranium Robertianum</i> .....	7/12, 8:30 A.M.	6.0	15.5	-61.3
	7/12, 8:30 A.M.	6.0	15.5	-61.3
	7/13, 2:30 P.M.	6.5	23.5	-72.3
<i>Chelidonium majus</i> .....	7/13, 2:30 P.M.	5.5	23.5	-76.6
<i>Rubus insericatus</i> .....	7/17, 8:15 A.M.	6.5	14.0	-53.6
<i>Campanula Rapunculus</i> .....	7/17, 4 P.M.	13.5	16.0	-15.6
	7/18, 8:15 A.M.	6.0	7.0	-14.3
<i>Galium silvaticum</i> .....	7/21, 8:15 A.M.	7.5	5.0	+50.0
<i>Campanula Trachelium</i> .....	8/20, 8:15 A.M.	16.0	18.0	-11.1
<i>Geranium Robertianum</i> .....	9/ 2, 1:30 P.M.	2.5	9.5	-73.7

\* Perigone.

TABLE 45. *Running Water (Roots and Stem in Water)*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Trifolium pratense</i> . . . . .	9/17, 5 P.M.	6.0	8.5	-29.4
<i>Solanum nigrum</i> . . . . .	9/27, 9:45 A.M.	5.5	9.5	-42.1
	9/28, 9:30 A.M.	6.5	7.5	-13.3
	9/28, 2:30 P.M.	8.0	9.0	-11.1
<i>Borago officinalis</i> . . . . .	9/14, 1:45 P.M.	5.5	12.0	-54.2
<i>Epilobium roseum</i> . . . . .	10/ 2, 9:15 A.M.	6.0	8.0	-25.0
<i>Bellis perennis</i> . . . . .	10/ 5, 9:15 A.M.	5.5	5.5	0.0

TABLE 46. *Stagnant Water (Roots and Stem in Water)*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Iris Pseudacorus</i> . . . . .	6/15, 9:20 A.M.	6.5	8.5	-23.5
<i>Comarum palustre</i> . . . . .	6/15, 9:20 A.M.	7.5	8.5	-11.8
<i>Veronica Beccabunga</i> . . . . .	6/19, 8:15 A.M.	6.5	4.0	+62.5
<i>Nymphozanthus luteus</i> . . . . .	6/28, 9 A.M.	4.5	7.0	-35.7
<i>Comarum palustre</i> . . . . .	7/ 2, 8:45 A.M.	9.5	6.5	+46.2
<i>Alisma Plantago aquatica</i> * . . . .	7/19, 9 A.M.	<2.5	7.0	-64.3
<i>Lysimachia vulgaris</i> . . . . .	7/19, 9 A.M.	7.5	7.0	+ 7.1
<i>Nymphaea alba</i> . . . . .	7/27, 8:15 A.M.	4.5	9.5	-52.6
	7/27, 3 P.M.	4.5	13.0	-65.4

\* Petals covered with rain.

TABLE 47. *Clearing and Edge of Wood*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Helleborus foetidus</i> .....	5/30, 8:30 A.M.	10.5	6.0	+ 75.0
<i>Rubus idaeus</i> .....	6/11, 2:30 P.M.	9.0	11.0	- 18.2
<i>Rosa canina</i> .....	7/ 4, 9 A.M.	9.5	6.0	+ 58.3
<i>Lysimachia nemorum</i> .....	7/ 5, 8:15 A.M.	6.0	6.0	0.0
	7/ 7, 9:15 A.M.	6.0	10.5	- 42.9
	7/ 9, 8:15 A.M.	6.0	10.5	- 42.9
<i>Hypericum perforatum</i> .....	7/ 9, 8:15 A.M.	9.5	10.5	- 9.5
<i>Cicerbita muralis</i> .....	7/16, 10 A.M.	7.5	16.0	- 53.1
<i>Potentilla anserina</i> .....	7/17, 10:30 A.M.	8.0	16.0	- 50.0
<i>Cicerbita muralis</i> .....	7/17, 2:15 P.M.	9.5	18.0	- 47.2
<i>Potentilla anserina</i> .....	7/18, 4 P.M.	6.0	7.0	- 14.3
<i>Trifolium medium</i> .....	7/25, 8:15 A.M.	10.5	8.0	+ 31.3
	7/26, 8:15 A.M.	12.5	11.0	+ 13.6
	7/27, 8:15 A.M.	12.5	9.5	+ 31.6
	8/13, 8:15 A.M.	12.0	17.0	- 29.4
<i>Knautia silvatica</i> .....	8/13, 4:30 P.M.	9.0	22.0	- 59.1
<i>Trifolium medium</i> .....	8/14, 8:15 A.M.	12.0	15.5	- 22.6
<i>T. pratense</i> .....	8/14, 8:15 A.M.	11.0	15.5	- 29.0
	8/16, 8:15 A.M.	11.0	18.5	- 40.5
<i>Leontodon hispidus</i> .....	8/16, 8:15 A.M.	10.5	18.5	- 43.2
<i>Solidago Virga aurea</i> .....	8/16, 8:15 A.M.	9.0	18.5	- 51.4
<i>Salvia glutinosa</i> .....	8/16, 8:15 A.M.	9.0	18.5	- 51.4
<i>Angelica silvestris</i> .....	8/16, 8:15 A.M.	8.0	18.5	- 56.8
<i>Trifolium pratense</i> .....	8/17, 8:15 A.M.	10.5	14.0	- 25.0
	8/18, 8:15 A.M.	10.5	21.5	- 51.2
<i>Satureia vulgaris</i> .....	8/18, 8:15 A.M.	15.5	21.5	- 27.9
<i>Trifolium pratense</i> .....	8/20, 8:15 A.M.	9.5	18.0	- 47.2
	8/21, 8:15 A.M.	8.0	22.5	- 64.4
	8/22, 8:15 A.M.	7.5	11.0	- 31.8
	8/24, 8:15 A.M.	11.0	15.0	- 26.7
<i>Convolvulus sepium</i> .....	8/24, 4:30 P.M.	4.0	16.0	- 75.0
<i>Medicago sativa</i> .....	8/24, 4:30 P.M.	8.0	16.0	- 50.0
<i>Trifolium pratense</i> .....	8/25, 8:15 A.M.	7.5	11.0	- 31.8
<i>Convolvulus sepium</i> .....	8/25, 8:15 A.M.	3.5	11.0	- 68.2
<i>Campanula rotundifolia</i> .....	8/25, 8:15 A.M.	8.0	11.0	- 27.3
<i>Trifolium pratense</i> .....	8/27, 8:15 A.M.	9.5	9.0	+ 5.6
<i>Epilobium montanum</i> .....	8/27, 8:15 A.M.	9.0	9.0	0.0
<i>Trifolium pratense</i> .....	8/28, 8:15 A.M.	6.5	8.0	- 18.8
<i>Convolvulus sepium</i> .....	8/28, 8:15 A.M.	4.0	8.0	- 50.0
<i>Trifolium pratense</i> .....	8/29, 8:15 A.M.	9.0	6.0	+ 50.0
	8/31, 8:15 A.M.	9.5	10.5	- 9.5
<i>Euphrasia Rostkoviana</i> .....	8/31, 8:15 A.M.	4.5	10.5	- 57.1
<i>Campanula rotundifolia</i> .....	8/31, 8:15 A.M.	9.0	10.5	- 14.3
<i>Trifolium pratense</i> .....	9/ 3, 8:15 A.M.	8.0	5.0	+ 60.0
<i>Epilobium montanum</i> .....	9/ 3, 9 A.M.	9.0	5.5	+ 63.6

TABLE 47 (continued)

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Trifolium pratense</i> .....	9/ 5, 8:15 A.M.	7.5	7.0	+ 7.1
	9/ 6, 8:15 A.M.	7.5	6.0	+ 25.0
<i>T. repens</i> .....	9/ 6, 2:45 P.M.	7.5	10.0	- 25.0
<i>T. pratense</i> .....	9/ 7, 8:15 A.M.	9.0	5.5	+ 63.6
<i>Potentilla anserina</i> .....	9/ 7, 10:30 A.M.	6.5	7.5	- 13.3
<i>Trifolium pratense</i> .....	9/ 8, 8:15 A.M.	9.5	7.0	+ 35.7
	9/10, 8:15 A.M.	10.5	5.0	+110.0
	9/11, 8:15 A.M.	10.5	9.0	+ 16.7
	9/11, 1:30 P.M.	12.0	13.0	- 7.7
	9/12, 8:15 A.M.	10.5	9.5	+ 10.5
	9/13, 8:15 A.M.	9.5	8.5	+ 11.8
	9/14, 8:15 A.M.	9.5	9.0	+ 5.6
<i>Potentilla anserina</i> .....	9/14, 8:15 A.M.	9.0	9.0	0.0
<i>Trifolium pratense</i> .....	9/15, 8:15 A.M.	11.0	5.0	+120.0
	9/17, 8:15 A.M.	7.5	6.0	+ 25.0
	9/18, 8:15 A.M.	7.5	6.0	+ 25.0
	9/19, 8:15 A.M.	6.5	5.0	+ 30.0
<i>Satureia vulgaris</i> .....	9/19, 9:15 A.M.	7.5	6.0	+ 25.0
<i>Trifolium pratense</i> .....	9/20, 8:15 A.M.	6.5	7.5	- 13.3
<i>Satureia vulgaris</i> .....	9/20, 10 A.M.	7.5	7.5	0.0
<i>Trifolium pratense</i> .....	9/21, 8:15 A.M.	6.0	5.5	+ 9.1
	9/22, 8:15 A.M.	6.0	7.0	- 14.3
	9/24, 8:15 A.M.	5.5	5.0	+ 10.0
	9/25, 8:15 A.M.	6.5	4.0	+ 62.5
	9/27, 8:15 A.M.	5.5	9.0	- 38.9
	9/28, 8:15 A.M.	6.5	7.5	- 13.3
	9/29, 8:15 A.M.	6.0	4.0	+ 50.0
	10/ 1, 9:15 A.M.	6.5	5.5	+ 18.2
	10/ 2, 8:15 A.M.	7.5	7.0	+ 7.1
<i>Epilobium montanum</i> .....	10/ 2, 9:15 A.M.	6.5	8.0	- 18.8
<i>Trifolium pratense</i> .....	10/ 3, 8:15 A.M.	6.5	6.0	+ 8.3
<i>Epilobium montanum</i> .....	10/ 3, 2:30 P.M.	6.5	7.0	- 7.1
<i>Geum urbanum</i> .....	10/ 4, 9:15 A.M.	10.5	7.0	+ 50.0
<i>Trifolium pratense</i> .....	10/ 5, 8:15 A.M.	6.0	8.0	- 25.0
<i>Geum urbanum</i> .....	10/ 5, 9:15 A.M.	7.5	8.5	- 11.8

TABLE 48. *Railroad and Wayside*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Cichorium Intybus</i> .....	7/16, 10 A.M.	12.0	16.0	- 25.0
<i>Convolvulus arvensis</i> .....	7/16, 10 A.M.	8.0	16.0	- 50.0
<i>Cichorium Intybus</i> .....	7/16, 2:30 P.M.	9.5	19.5	- 51.3
<i>Achillea Millefolium</i> .....	7/23, 2:15 P.M.	16.0	8.0	+100.0
<i>Lotus corniculatus</i> .....	8/14, 4 P.M.	19.5	19.5	0.0
<i>Sisymbrium officinale</i> .....	8/14, 4 P.M.	17.5	19.5	- 10.3
<i>Daucus Carota</i> .....	8/14, 4 P.M.	19.5	19.5	0.0
<i>Centaurea Jacea</i> .....	8/16, 2:30 P.M.	15.5	24.0	- 35.4
<i>Leontodon autumnalis</i> .....	8/16, 2:30 P.M.	16.0	24.0	- 33.3
<i>Verbena officinalis</i> .....	8/17, 4 P.M.	12.0	19.0	- 36.8
<i>Linaria vulgaris</i> .....	8/18, 4 P.M.	23.5	21.0	+ 11.9
<i>Silene vulgaris</i> .....	8/18, 4 P.M.	15.5	21.0	- 26.2
<i>Melilotus albus</i> .....	8/20, 4 P.M.	6.5	21.0	- 69.0
<i>Echium vulgare</i> .....	8/20, 4 P.M.	6.5	21.0	- 69.0
<i>Senecio viscosus</i> .....	8/21, 4:20 P.M.	12.5	28.0	- 55.4
<i>Rapistrum rugosum</i> .....	8/21, 4:20 P.M.	10.5	28.0	- 62.5
<i>Erucastrum nasturtifolium</i> .....	8/29, 8:15 A.M.	15.5	6.0	+158.3
<i>Melandrium album</i> .....	8/29, 5 P.M.	8.0	7.5	+ 6.7
<i>Geranium pyrenaicum</i> .....	9/ 3, 4:15 P.M.	9.5	7.5	+ 26.7
<i>Sonchus oleraceus</i> .....	9/ 5, 3:15 P.M.	6.5	10.0	- 35.0
<i>Cirsium arvense</i> .....	9/ 5, 3:15 P.M.	11.0	10.0	+ 10.0
<i>Sonchus oleraceus</i> .....	9/ 6, 9:30 A.M.	12.0	7.0	+ 71.4
<i>Cirsium arvense</i> .....	9/ 6, 9:30 A.M.	11.0	7.0	+ 57.1
<i>Linaria minor</i> .....	9/ 6, 9:30 A.M.	8.0	7.0	+ 14.3
	9/ 6, 2:45 P.M.	9.5	10.0	- 5.0
<i>Trifolium repens</i> .....	9/ 6, 2:45 P.M.	9.5	10.0	- 5.0
<i>Bellis perennis</i> .....	9/ 6, 2:45 P.M.	11.0	10.0	+ 10.0
<i>Potentilla anserina</i> .....	9/ 7, 10:30 A.M.	12.0	7.5	+ 60.0
<i>Oxalis stricta</i> .....	9/ 7, 2:30 P.M.	13.5	9.0	+ 50.0
<i>Anagallis arvensis</i> .....	9/ 7, 2:30 P.M.	12.5	9.0	+ 38.9
<i>Melilotus officinalis</i> .....	9/ 7, 2:30 P.M.	14.5	9.0	+ 61.1
<i>Linum usitatissimum</i> .....	9/ 8, 9:30 A.M.	6.5	8.5	- 23.5
<i>Verbascum Thapsus</i> .....	9/ 8, 10:15 A.M.	8.0	9.0	- 11.1
<i>Trifolium pratense</i> .....	9/11, 8:15 A.M.	9.5	9.0	+ 5.6
	9/11, 1:30 P.M.	11.0	13.0	- 15.4
<i>Stellaria aquatica</i> .....	9/11, 1:30 P.M.	17.0	13.0	+ 30.8
<i>Potentilla anserina</i> .....	9/14, 8:15 A.M.	9.0	9.0	0.0
<i>Melilotus albus</i> .....	9/15, 4 P.M.	6.0	7.0	- 14.3
<i>Linaria vulgaris</i> .....	9/21, 4 P.M.	8.0	5.5	+ 45.5
<i>Stellaria aquatica</i> .....	9/21, 4 P.M.	6.5	5.5	+ 18.2
<i>Solanum nigrum</i> .....	9/28, 9:30 A.M.	5.5	7.5	- 26.7
	9/28, 2:30 P.M.	6.0	9.0	- 33.3
<i>Echium vulgare</i> .....	9/29, 1:30 P.M.	9.0	8.0	+ 12.5
<i>Melilotus albus</i> .....	9/29, 1:30 P.M.	9.5	8.0	+ 18.8
<i>Hypericum perforatum</i> .....	9/29, 1:30 P.M.	9.5	8.0	+ 18.8
<i>Potentilla reptans</i> .....	10/ 1, 4 P.M.	12.5	10.0	+ 25.0

TABLE 49. *Wall, and Molasse and Conglomerate Boulders*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Geranium Robertianum</i> .....	6/25, 2 P.M.	5.5	10.0	- 45.0
<i>Hieracium murorum</i> .....	7/11, 8:30 A.M.	14.5	15.5	- 6.5
		15.5	15.5	0.0
<i>Chrysanthemum Leucanthemum</i> .	7/11, 8:30 A.M.	16.0	15.5	+ 3.2
		16.0	15.5	+ 3.2
<i>Geranium Robertianum</i> .....	7/12, 8:30 A.M.	8.0	15.5	- 46.7
<i>Chelidonium majus</i> .....	7/13, 2:30 P.M.	9.5	23.5	- 59.6
<i>Geranium Robertianum</i> .....	7/13, 2:30 P.M.	6.5	23.5	- 72.3
<i>Campanula rotundifolia</i> .....	7/23, 2:15 P.M.	15.5	8.0	+ 93.8
<i>Linaria Cymbalaria</i> .....	9/10, 2:45 P.M.	16.0	8.0	+100.0
<i>Convolvulus sepium</i> .....	9/10, 2:45 P.M.	14.5	8.0	+ 81.3
<i>Saponaria officinalis</i> .....	9/13, 2:30 P.M.	12.5	13.0	- 3.8
<i>Scabiosa columbaria</i> .....	9/14, 1:45 P.M.	21.5	12.0	+ 79.2
	9/21, 2:30 P.M.	5.5	5.5	0.0
<i>Geranium Robertianum</i> .....	10/ 1, 1:45 P.M.	9.0	11.0	- 18.2
	10/ 2, 1:30 P.M.	6.0	9.5	- 36.8

TABLE 50. *Molasse Cliff*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Saxifraga aizoides</i> .....	8/27, 4:30 P.M.	12.0	12.5	- 4.0
<i>Ononis spinosa</i> .....	8/27, 4:30 P.M.	12.5	12.5	0.0
<i>Saxifraga aizoides</i> .....	9/ 3, 3 P.M.	8.0	8.0	0.0
<i>Campanula cochleariifolia</i> .....	9/ 3, 3 P.M.	11.0	8.0	+37.5
<i>Parnassia palustris</i> .....	9/15, 2:45 P.M.	6.0	7.0	-14.3

TABLE 51. *River Sand*

Name of Plant	Date, 1923	Suction Force of Corolla in Atm.		Difference (%) Compared with Bellis
			Bellis Control	
<i>Melilotus albus</i> .....	9/15, 2:45 P.M.	6.5	7.5	- 13.3
<i>Saponaria officinalis</i> .....	9/17, 5 P.M.	6.5	8.5	- 23.5
<i>Chrysanthemum Leucanthemum</i> .	9/17, 5 P.M.	4.5	8.5	- 47.1
<i>Erucastrum nasturtiiifolium</i> .....	9/18, 5 P.M.	9.5	8.5	+ 11.8
<i>Aster versicolor</i> .....	9/18, 5 P.M.	7.5	8.5	- 11.8
<i>Gypsophila repens</i> .....	9/19, 9:15 A.M.	13.5	6.0	+125.0
<i>Salvia pratensis</i> .....	9/19, 9:15 A.M.	12.0	6.0	+100.0

After having reckoned out the mean suction force for the plants of each given habitat, the corresponding mean of Bellis was placed next to it. The result is given in table 52.

TABLE 52

Habitat	Number of Measurements	Suction Force of Corollas in Atm.	Corresponding Mean of <i>Bellis</i> Corolla in Atm.	Difference (%) Compared with <i>Bellis</i>
Fresh water pond (plant, excluding flowers, submersed).....	1	4.0	8.5	-52.9
Humid forest and hedgerow.....	33	6.9	10.4	-33.7
Running water; roots and stem in water.....	7	6.1	8.6	-29.1
Stagnant water; roots and stem in water.....	9	5.9	7.9	-25.3
Clearing and edge of wood.....	80	8.4	10.3	-18.4
Railroad and wayside.....	46	11.3	12.6	-10.3
Wall, molasse and conglomerate boulders.....	16	12.0	13.1	- 8.3
Molasse cliff.....	5	9.9	9.6	+ 3.1
River sand.....	7	8.6	7.6	+13.2

As table 52 shows, the submersed aquatic plants have the lowest mean suction force. This can be readily understood. It is also evident that the other aquatic plants and the plants growing in the protected wood should have a low mean suction force.

It is less clear that plants growing on walls, molasse boulders, and railroad beds, which ordinarily are considered as dry habitats, should be represented by negative percentages, as against the control *Bellis*, which grew in a garden bed. The reason is a double one: first, *Bellis* grew in a plot which was not watered during the whole period of experimentation; again, it grew at the edge of an elevated bed, so that its soil readily lost its moisture during the dry summer of 1923. Once more, the plants growing in the drier habitats were protected against too great disturbances in their water balance by deep root systems and by other still imperfectly known economics.

In the few cases in which I examined different portions of the same flower, the lowest suction force was found in the innermost organs and higher suction forces progressively outward.

In *Helleborus foetidus*, the lower foliar epidermis of the midveins of the leaflets shows a higher suction force at the apex (11.0 atm.) than at the base (9.0 atm.). The middles of the midveins of different leaflets show the same suction forces, with the exception of those of the outermost leaflets, whose middles, being nearer the petioles, show lower suction forces. This comparison is shown by the following figures: Leaflet 1, suction force at middle of midvein 11 atm., leaflet 3, 11 atm., leaflet 4, 9.5 atm.; leaflet 1, 9.5 atm., leaflet 5, 9.0 atm.; leaflet 1, 10.5 atm., leaflet 2, 10.5 atm.

The epidermis at the bottom of the stalk (1 cm. high) possessed a suction force of 6.5 atm., that of the upper portion (15 cm. high) 9.0 atm.

In six cases in which the corollas and root tips of the same plants were measured, the former were always undoubtedly higher. *Lysimachia vulgaris* showed a suction force of 4.5 atm. in the root tips and 7.5 atm. in the corolla.

AGGREGATE MEASUREMENTS ARRANGED ACCORDING TO FAMILIES  
TABLE 53

Family,* Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
Typhaceae <i>Typha latifolia</i> L.....	Stagnant water	Root tips Root tips	6/26/23, 8:30 A.M. 6/27/23, 9 A.M.	4.5 4.5	7.0 7.0
Sparganiaceae <i>Sparganium minimum</i> Fries.....	Stagnant water	{ Filaments Root tips Filaments Root tips Root tips	{ 7/20/23, 8:45 A.M. 7/20/23, 8:45 A.M. 7/25/23, 8:15 A.M. 7/25/23, 8:15 A.M. 7/25/23, 8:15 A.M.	{ 5.5 2.5 6.0 4.5 2.5	{ 4.0 4.0 8.0 8.0 8.0
Potamogetonaceae <i>Potamogeton crispus</i> L.....	Fresh water	Root tips Leaf blade Root tips Leaf blade Root tips	6/22/23, 8:15 A.M. 6/27/23, 9 A.M. 2/28/24, 8:15 A.M. 2/28/24, 8:15 A.M. 2/29/24, 8:15 A.M.	4.0 6.0 2.5 4.5 3.5	9.5 7.0
Alismataceae <i>Alisma Plantago aquatica</i> L.....	Stagnant water	Corolla (full of rain)	7/19/23, 9 A.M.	<2.5	7.0
Hydrocharitaceae <i>Elodea canadensis</i> Michaux.....	Fresh water	Root tips Root tips Leaf blades Root tips Leaf blades	6/20/23, 8:30 A.M. 6/22/23, 8:15 A.M. 6/22/23, 8:15 A.M. 6/25/23, 8:30 A.M. 2/28/24, 8:15 A.M.	4.0 4.0 4.5 3.5 4.0	8.5 9.0 9.5 7.5
Juncaceae <i>Juncus nemorosa</i> (Pall.) E. Meyer	Humid forest	Styles	6/18/23, 2:15 P.M.	7.5	7.0
Liliaceae <i>Maianthemum bifolium</i> (L.) F. W. Schmidt.....	Humid forest	{ Perigone Filaments	{ 6/ 5/23, 2:30 P.M. 6/ 5/23, 2:30 P.M.	{ 6.0 5.5	{ 7.0 7.0
Iridaceae <i>Iris Pseudacorus</i> L.....	Stagnant water	Outer perigone	6/15/23, 9:20 A.M.	6.5	8.5

\* All plant names were checked by Dr. F. Jacquet. The nomenclature is taken from Schinz and Keller, "Flora der Schweiz," 4th edition.



TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
Orchidaceae					
† <i>Platanthera bifolia</i> (L.) Rich. ....	Humid forest	Outer perigone	7/ 4/23, 9 A.M.	5.5	6.0
<i>Cephalanthera rubra</i> (L.) Rich. ....	Humid forest	Outer perigone	7/ 4/23, 9 A.M.	9.0	6.0
<i>Neottia Nidus-avis</i> (L.) Rich. ....	Humid forest	Outer perigone	6/21/23, 10 A.M.	4.5	11.0
		Root, cross sections	6/21/23, 10 A.M.	3.5	11.0
		Root, median longitudinal sections	6/21/23, 10 A.M.	4.0	11.0
Caryophyllaceae					
<i>Silene vulgaris</i> (Monch.) Garcke .	Wayside	Corolla	8/18/23, 4 P.M.	15.5	21.0
<i>Melandrium album</i> (Miller) Garcke. ....	Wayside	Corolla	8/29/23, 5 P.M.	8.0	7.5
<i>Gypsophila repens</i> L. ....	River sand	Corolla	9/19/23, 9:15 A.M.	13.5	6.0
<i>Saponaria officinalis</i> (L.) ....	Conglomerate boulder Moist river sand, stem in water	Corolla	9/13/23, 2:30 P.M.	12.5	13.0
	Wayside	Corolla	9/17/23, 5 P.M.	6.5	8.5
<i>Stellaria aquatica</i> (L.) Scop. ....		Corolla	9/11/23, 1:30 P.M.	17.0	13.0
		Corolla	9/21/23, 4 P.M.	8.0	5.5
Nymphaeaceae					
<i>Nymphaea alba</i> L. ....	Stagnant water	Corolla	8/27/23, 8:15 A.M.	4.5	9.5
<i>Nymphaeodanthus luteus</i> (L.) Fernald. ....	Stagnant water	Corolla	8/27/23, 3 P.M.	4.5	13.0
Ranunculaceae					
<i>Helleborus foetidus</i> L. ....	Edge of wood	Corolla	6/28/23, 9 A.M.	4.5	7.0
		Lower foliar epidermis over apex of midvein, leaflet 1 †	5/14/23, 3 P.M.	11.0	Same plant
		Similar to above, but at base of midvein. L.1	5/14/23, 3 P.M.	9.0	

† *Platanthera* and *Cephalanthera* were equal in height and grew next to each other.† *Helleborus foetidus* has pedate leaves. For convenience' sake I numbered the middle leaflet (L.1), the ones to the right and left of it (L.2), etc.

TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
<i>Helleborus foetidus</i> L.....	Edge of wood	Similar to first, but at middle of midvein. L.1	5/15/23, 8:15 A.M.	9.5	
		L.5	5/15/23, 8:15 A.M.	9.0	
		L.1	5/15/23, 4:15 P.M.	10.5	
		L.2	5/15/23, 4:15 P.M.	10.5	
		L.1	5/16/23, 8:30 A.M.	11.0	
		L.3	5/16/23, 8:30 A.M.	11.0	
		L.4	5/16/23, 8:30 A.M.	9.5	
		L.1	5/16/23, 3:45 P.M.	8.0	
		{Epidermis of petiole on lower side, upper end	5/16/23, 3:45 P.M.	7.5	
		Same, but at lower end	5/16/23, 3:45 P.M.	7.5	
		Lower foliar epidermis over base of midvein, leaflet 1	5/18/23, 8:30 A.M.	10.5	
		Epidermis of petiole on upper side, lower end	5/18/23, 8:30 A.M.	6.5	
		Parenchyma of petiole, lower end, upper side	5/18/23, 2:30 P.M.	14.5	
		{Epidermis of stalk, 1 cm. high	5/23/23, 8:45 A.M.	6.5	
		Epidermis of stalk, 15 cm. high	5/23/23, 8:45 A.M.	9.0	
		Lower foliar epidermis over base of midvein (L.1)	5/23/23, 2:30 P.M.	11.0	
		Innermost pith (stalk), 15 cm. high	5/23/23, 2:30 P.M.	14.0	
		Outermost pith (stalk), 15 cm. high		16.0	
		Cortical parenchyma (stalk), 15 cm. high		14.0	
		Epidermis of stalk, 1 cm. high		8.0	
		Lower foliar epidermis over base of midvein (L.1)	5/24/23, 8:30 A.M.	12.0	
		Epidermis of stalk, 1 cm. high		7.5	
		Inner pith (stalk), 1 cm. high		10.5	
		Outer pith (stalk), 1 cm. high		11.0	



TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
<i>Ranunculus flaccidus</i> Pers.....	Fresh water	Corolla Leaflets¶ Root tips Root tips	6/20/23, 8:30 A.M. 6/20/23, 8:30 A.M. 6/22/23, 8:30 A.M. 6/27/23, 8:30 A.M. 2/29/24, 8:15 A.M. 2/29/24, 8:15 A.M. 6/13/23, 8:15 A.M. 7/13/23, 2:30 P.M. 7/13/23, 2:30 P.M.	4.0 7.5 4.5 4.0 2.5 4.0 4.5 5.5 9.5	8.5 8.5 9.5 7.0  7.5 23.5 23.5
Papaveraceae					
<i>Chelidonium majus</i> L.....	Hedgerow Hedgerow Wall	Corolla Corolla Corolla	6/13/23, 8:15 A.M. 7/13/23, 2:30 P.M. 7/13/23, 2:30 P.M.	4.5 5.5 9.5	7.5 23.5 23.5
Cruciferae					
<i>Sisymbrium officinale</i> (L.) Scop...	Railroad	Corolla	8/14/23, 4 P.M.	17.5	19.5
<i>Erucastrum nasturtifolium</i> (Poir.) O. E. Schulz.....	Railroad River sand Wayside	Corolla Corolla Corolla	8/29/23, 9:15 A.M. 9/18/23, 5 P.M. 8/21/23, 4:20 P.M.	15.5 9.5 10.5	6.0 8.5 28.0
<i>Rapistrum rugosum</i> (L.) All.....	Molasse cliff	Corolla	8/27/23, 4:30 P.M.	12.0	12.5
Saxifragaceae					
<i>Saxifraga aizoides</i> L.....	Molasse cliff	Corolla	9/ 3/23, 3 P.M. 9/15/23, 2:45 P.M.	8.0 6.0	8.0 7.0
Rosaceae					
<i>Rubus idaeus</i> L.....	Edge of wood	Corolla	6/11/23, 2:30 P.M.	9.0	11.0
<i>R. insarcticus</i> Ph. J. Müller.....	Humid forest	Corolla	7/17/23, 8:15 A.M.	6.5	14.0
<i>Fragaria vesca</i> L.....	Humid forest	Corolla	6/14/23, 8:15 A.M.	4.5	6.0
<i>Comarum palustre</i> L.....	Stagnant water	Corolla Root tips Root tips Root tips	6/15/23, 9:20 A.M. 6/15/23, 9:20 A.M. 7/ 2/23, 8:45 A.M. 7/ 2/23, 8:45 A.M.	7.5 4.5 9.5 6.5	8.5 8.5 6.5 6.5
<i>Potentilla reptans</i> L.....	Railroad	Root tips	8/ 3/23, 7:45 A.M.	4.5	7.0
<i>P. anserina</i> L.....	Edge of wood	Corolla	10/ 1/23, 4 P.M. 7/17/23, 10:30 A.M. 7/18/23, 4 P.M.	12.5 8.0 6.0	10.0 16.0 7.0
	Railroad	Corolla	9/ 7/23, 10:30 A.M. 9/ 7/23, 10:30 A.M. 9/14/23, 8:15 A.M. 9/14/23, 8:15 A.M.	6.5 12.0 9.0 9.0	7.5 7.5 9.0 9.0

¶ Different leaflets of the same leaf showed great differences in suction force.

TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
<i>Geum urbanum</i> L.....	Edge of wood	Corolla	10/ 4/23, 9:15 A.M.	10.5	7.0
<i>Rosa arvensis</i> Hudson.....	Humid forest	Corolla	10/ 5/23, 9:15 A.M. 7/ 5/23, 8:15 A.M. 7/ 7/23, 9:15 A.M.	7.5 10.5 9.5	8.5 6.0 6.0
<i>R. canina</i> L. var. <i>luteitana</i> (Leman) Baker.....	Wood, clearing	Corolla	7/ 4/23, 9 A.M.	9.5	6.0
Leguminosae					
<i>Ononis spinosa</i> L.....	Molasse cliff	Standard of corolla	8/27/23, 4:30 P.M.	12.5	12.5
<i>Medicago sativa</i> L.....	Edge of wood	Standard of corolla	8/24/23, 4:30 P.M.	8.0	16.0
<i>Melilotus albus</i> Desr.....	Railroad	Standard of corolla	8/20/23, 4 P.M.	6.5	21.0
	River sand		9/15/23, 4 P.M.	6.0	7.0
	Railroad		9/15/23, 4 P.M.	6.5	7.5
	Railroad		9/29/23, 1:30 P.M.	9.0	8.0
<i>M. officinalis</i> (L.) Lam.....	Railroad	Standard of corolla	9/ 7/23, 2:30 P.M.	14.5	9.0
<i>Trifolium medium</i> Hudson.....	Edge of wood	Standard of corolla	7/25/23, 8:15 A.M. 7/26/23, 8:15 A.M. 7/27/23, 8:15 A.M. 8/13/23, 8:15 A.M. 8/14/23, 8:15 A.M. 8/14/23, 8:15 A.M. 8/16/23, 8:15 A.M. 8/17/23, 8:15 A.M. 8/18/23, 8:15 A.M. 8/20/23, 8:15 A.M. 8/21/23, 8:15 A.M. 8/22/23, 8:15 A.M. 8/24/23, 8:15 A.M. 8/25/23, 8:15 A.M. 8/27/23, 8:15 A.M. 8/28/23, 8:15 A.M. 8/29/23, 8:15 A.M. 8/31/23, 8:15 A.M. 9/ 3/23, 8:15 A.M. 9/ 5/23, 8:15 A.M. 9/ 6/23, 8:15 A.M.	10.5 12.5 12.5 12.0 12.0 11.0 11.0 10.5 10.5 9.5 8.0 7.5 11.0 15.0 11.0 9.0 8.0 6.0 10.5 5.0 7.0 7.5	11.0 17.0 15.5 15.5 18.5 14.0 21.5 18.0 22.5 11.0 15.0 11.0 9.0 8.0 6.0 10.5 5.0 7.0 6.0
<i>T. pratense</i> L.....	Edge of wood	Standard of corolla			

TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bells in Atm.
<i>T. pratense</i> L.....	Edge of wood	Standard of corolla	9/ 7/23, 8:15 A.M.	9.0	5.5
			9/ 8/23, 8:15 A.M.	9.5	7.0
			9/10/23, 8:15 A.M.	10.5	5.0
	Edge of wood Wayside	Standard of corolla	9/11/23, 8:15 A.M.	10.5	9.0
			9/11/23, 8:15 A.M.	9.5	9.0
			9/11/23, 1:30 P.M.	12.0	13.0
	Edge of wood	Standard of corolla	9/11/23, 1:30 P.M.	11.0	13.0
			9/12/23, 8:15 A.M.	10.5	9.5
			9/13/23, 8:15 A.M.	9.5	8.5
			9/14/23, 8:15 A.M.	9.5	9.0
<i>T. pratense</i> L.....	Running water	Standard of corolla	9/15/23, 8:15 A.M.	11.0	5.0
			9/17/23, 8:15 A.M.	7.5	6.0
			9/17/23, 5 P.M.	6.0	8.5
	Edge of wood	Standard of corolla	9/18/23, 8:15 A.M.	7.5	6.0
			9/19/23, 8:15 A.M.	6.5	5.0
			9/20/23, 8:15 A.M.	6.5	7.5
	Edge of wood	Standard of corolla	9/21/23, 8:15 A.M.	6.0	5.5
			9/22/23, 8:15 A.M.	6.0	7.0
			9/24/23, 8:15 A.M.	5.5	5.0
			9/25/23, 8:15 A.M.	6.5	4.0
<i>T. repens</i> L.....	Edge of wood	Standard of corolla	9/27/23, 8:15 A.M.	5.5	9.0
			9/28/23, 8:15 A.M.	6.5	7.5
			9/29/23, 8:15 A.M.	6.0	4.0
	Edge of wood Railroad Wayside	Standard of corolla	10/ 1/23, 8:15 A.M.	6.5	5.5
			10/ 2/23, 8:15 A.M.	7.5	7.0
			10/ 3/23, 8:15 A.M.	6.5	6.0
	Wayside	Standard of corolla	10/ 5/23, 8:15 A.M.	6.0	8.0
			9/ 6/23, 2:45 P.M.	7.5	10.0
			8/14/23, 4 P.M.	19.5	19.5
			9/ 3/23, 4:15 P.M.	9.5	7.5
<i>Geranium pyrenaicum</i> Burm.....	Wayside	Corolla			

TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
<i>G. Robertianum</i> L.....	Humid forest	Corolla	6/12/23, 4 P.M.	4.5	9.5
	Humid forest	Corolla	6/25/23, 2 P.M.	5.5	10.0
	Wall	Corolla	6/25/23, 2 P.M.	5.5	10.0
	Humid forest	Corolla	7/12/23, 8:30 A.M.	6.0	15.5
	Wall	Corolla	7/12/23, 8:30 A.M.	6.0	15.5
	Humid forest	Corolla	7/12/23, 8:30 A.M.	8.0	15.5
	Wall	Corolla	7/13/23, 2:30 P.M.	6.5	23.5
	Humid forest	Corolla	7/13/23, 2:30 P.M.	6.5	23.5
	Wall	Corolla	9/ 2/23, 1:30 P.M.	2.5	9.5
	Wall	Corolla	10/ 1/23, 1:45 P.M.	9.0	11.0
	Wall	Corolla	10/ 2/23, 1:30 P.M.	6.0	9.5
Linaceae					
<i>Linum usitatissimum</i> L.....	Wayside	Corolla	9/ 8/23, 9:30 A.M.	6.5	8.5
Oxalidaceae					
<i>Oxalis stricta</i> L.....	Wayside	Corolla	9/ 7/23, 2:30 P.M.	13.5	9.0
Hypericaceae					
<i>Hypericum perforatum</i> L.....	Edge of wood	Corolla	7/ 9/23, 8:15 A.M.	9.5	10.5
	Wayside	Corolla	9/29/23, 1:30 P.M.	9.5	8.0
Oenotheraceae					
<i>Epilobium angustifolium</i> L.....	Protected mixed wood	Corolla	7/10/23, 8:15 A.M.	6.0	13.5
		Corolla	7/10/23, 4:15 P.M.	8.0	17.5
	Humid forest	Corolla	7/ 2/23, 3 P.M.	8.0	8.0
<i>E. montanum</i> L.....	Edge of wood	Corolla	7/27/23, 3 P.M.	9.0	9.0
			9/ 3/23, 9 A.M.	9.0	5.5
		Roots	10/ 2/23, 9:15 A.M.	6.5	8.0
		Roots	10/ 2/23, 4:45 P.M.	6.5	9.5
		Roots	10/ 3/23, 8:15 A.M.	5.5	6.0
		Corolla	10/ 3/23, 2:30 P.M.	5.5	7.0
<i>E. roseum</i> Schreber.....	Running water	Corolla	10/ 3/23, 2:30 P.M.	6.5	7.0
		Roots	10/ 2/23, 9:15 A.M.	6.0	8.0
		Roots	10/ 2/23, 4:45 P.M.	6.0	9.5

TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
Halorrhagidaceae <i>Myriophyllum verticillatum</i> L. §...	Fresh water	Root tips Leaflets Root tips Leaflets	7/ 3/23, 7:45 A.M. 7/20/23, 8:45 A.M. 7/24/23, 8:45 A.M. 7/24/23, 8:45 A.M. 6/15/23, 9:20 A.M. 6/15/23, 9:20 A.M.	4.5 7.5 5.5 6.5 6.0 4.5	7.0 4.0 4.0 4.0 8.5 8.5
Araliaceae <i>Hedera Helix</i> L.....	Edge of wood	Leaf blade Lower foliar epidermis over base of midvein	2/26/24, 2:15 P.M. 2/26/24, 2:15 P.M.	71.5 24.5	
Umbelliferae <i>Angelica silvestris</i> L..... <i>Daucus Carota</i> L.....	Edge of wood Wayside	Corolla Corolla	8/16/23, 8:15 A.M. 8/14/23, 4 P.M.	8.0 19.5	18.5 19.5
Primulaceae <i>Lysimachia vulgaris</i> L..... <i>L. nemorum</i> L.....	Stagnant water Edge of wood	Corolla Root tips Corolla	7/19/23, 9 A.M. 7/19/23, 9 A.M. 7/ 5/23, 8:15 A.M. 7/ 7/23, 9:15 A.M. 7/ 9/23, 8:15 A.M.	7.5 4.5 6.0 6.0 6.0	7.0 7.0 6.0 10.5 10.5
Apocynaceae <i>Anagallis arvensis</i> L. subsp. <i>phoenicia</i> (Scop.) Schinz & Keller..... <i>Vinca minor</i> L.....	Railroad Edge of wood	Corolla Lower foliar epidermis over base of midvein	9/ 7/23, 2:30 P.M. 6/ 8/23, 2:30 P.M. 6/29/23, 8:15 A.M. 7/ 2/23, 2:15 P.M. 7/ 3/23, 2:15 P.M. 7/ 4/23, 2:15 P.M. 7/ 7/23, 2:15 P.M. 2/27/24, 8:15 A.M.	12.5 7.5 8.0 8.0 9.0 9.0 10.5 23.5 62.0	9.0 9.5 7.5 8.0 13.0 8.0 13.0 13.0

§ Different leaflets of the same leaf often had very different suction forces.

|| Outermost florets.





TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bellis in Atm.
Scrophulariaceae					
<i>Verbascum Thapsus</i> L.....	Wayside	Corolla	9/ 8/23, 10:15 A.M.	8.0	9.0
<i>Linaria Cymbalaria</i> (L.) Miller ..	Wall	Corolla	9/10/23, 2:45 P.M.	16.0	8.0
<i>L. vulgaris</i> Miller.....	Railroad	Corolla	8/18/23, 4 P.M.	23.5**	21.0
<i>L. minor</i> (L.) Desf.....	Railroad	Corolla	9/21/23, 4 P.M.	8.0	5.5
<i>Veronica Beccabunga</i> L.....	Stagnant water	Corolla	9/ 6/23, 9:30 A.M.	8.0	7.0
			9/ 6/23, 2:45 P.M.	9.5	10.0
			6/19/23, 8:15 A.M.	9.5	4.0
			6/19/23, 8:15 A.M.	4.5	4.0
			6/19/23, 8:15 A.M.	5.5	4.0
			6/19/23, 8:15 A.M.	6.0	4.0
<i>V. Chamaedrys</i> L.....	Humid forest	Corolla	6/13/23, 4:45 P.M.	4.5	9.5
<i>V. officinalis</i> L.....	Humid forest	Corolla	6/18/23, 2:15 P.M.	6.5	7.0
<i>Euphrasia Roskoviana</i> Hayne....	Clearing of wood	Corolla	8/13/23, 8:15 A.M.	4.5	10.5
Rubiaceae					
<i>Asperula odorata</i> L.....	Humid forest	Corolla	6/ 6/23, 8:30 A.M.	5.5	7.5
<i>Galium silvaticum</i> (L.) Duby....	Humid forest	Corolla	6/ 6/23, 5:15 P.M.	5.5	8.5
Dipsaceae††					
<i>Knautia silvatica</i> (L.) Duby.....	Clearing of wood	Corolla	7/21/23, 8:15 A.M.	7.5	5.0
<i>Scabiosa columbaria</i> L.....	Molasse boulder	Corolla	8/13/23, 4:30 P.M.	9.0	22.0
			9/14/23, 1:45 P.M.	21.5	12.0
			9/21/23, 2:30 P.M.	5.5	5.5
Campanulaceae					
<i>Phyteuma spicatum</i> L.....	Humid forest	Corolla	6/12/23, 2:30 P.M.	7.5	10.5
<i>Campanula cochlearifolia</i> Lam....	Molasse cliff	Corolla	9/ 3/23, 3 P.M.	11.0	8.0
<i>C. rotundifolia</i> L.....	Wall	Corolla	7/23/23, 2:15 P.M.	15.5	8.0
	Edge of wood	Corolla	8/25/23, 8:15 A.M.	8.0	11.0
			8/31/23, 8:15 A.M.	9.0	10.5
<i>C. Rapunculus</i> L.....	Humid forest	Corolla	7/17/23, 4 P.M.	13.5	16.0
<i>C. Trachelium</i> L.....	Humid forest	Corolla	7/18/23, 8:15 A.M.	6.0	7.0
			8/20/23, 8:15 A.M.	16.0	18.0

\*\* Next to track.

†† The outer florets were taken.

TABLE 53 (continued)

Family, Genus, and Species	Habitat	Tissue Examined	Date	Suction Force in Atm.	Suction Force of Corolla of Control Bells in Atm.
Compositae††					
<i>Solidago Virga aurea</i> L. ....	Edge of wood	Corolla	8/16/23, 8:15 A.M.	9.0	18.5
<i>Bellis perennis</i> L. ....	Wayside	Corolla	9/ 6/23, 2:45 P.M.	11.0	10.0
	Running water	Corolla	10/ 5/23, 9:15 A.M.	5.5	5.5
<i>Aster versicolor</i> Willd. ....	River sand	Corolla	9/18/23, 5 P.M.	7.5	8.5
<i>Achillea Millefolium</i> L. ....	Railroad	Corolla	7/23/23, 2:15 P.M.	16.0	8.0
<i>Tussilago Farfara</i> L. ....	Edge of wood	Corolla	2/25/24, 8:15 A.M.	18.0	
			2/26/24, 8:15 A.M.	23.0	
			2/27/24, 8:15 A.M.	14.5	
			3/ 1/24, 8:15 A.M.	14.5	
<i>Senecio viscosus</i> L. ....	Edge of wood	Corolla	3/ 3/24, 9 A.M.	19.5	
<i>Cirsium arvense</i> (L.) Scop. ....	Wayside	Corolla	8/21/23, 4:20 P.M.	12.5	28.0
<i>Centaurea Jacea</i> L. ....	Railroad	Corolla	9/ 5/23, 3:15 P.M.	11.0	10.0
<i>Cichorium Intybus</i> L. ....	Railroad	Corolla	8/16/23, 2:30 P.M.	15.5	24.0
			7/16/23, 10 A.M.	12.0	16.0
<i>Leontodon autumnalis</i> L. ....	Railroad	Corolla	7/16/23, 2:30 P.M.	9.5	19.5
<i>L. hispidus</i> L. var. <i>glaberrimus</i> (Koch) Bischoff ( <i>L. hastilis</i> L.)	Clearing of wood	Corolla	8/16/23, 2:30 P.M.	16.0	24.0
<i>Cicerbita muralis</i> (L.) Wallr. ....	Edge of wood	Corolla	8/16/23, 2:30 P.M.	10.5	18.5
<i>Santhus oleraceus</i> L. em. Gouan. ....	Wayside	Corolla	7/16/23, 10 A.M.	7.5	16.0
			7/17/23, 2:15 P.M.	9.5	18.0
<i>Hieracium murorum</i> L. em. Hudson subsp. <i>circumstellatum</i> Zahn. ....	Humid forest	Corolla	9/ 5/23, 3:15 P.M.	12.0	7.0
			9/ 6/23, 11 A.M.	12.0	7.0
		Corolla	6/ 8/23, 5:30 P.M.	6.5	7.5
		Corolla	6/ 9/23, 11 A.M.	8.0	9.0
<i>H. murorum</i> L. em. Hudson subsp. <i>gentile</i> (Jordan). ....	Conglomerate boulder	Lower foliar epidermis over base of midvein of lowest leaf of rosette	6/ 9/23, 11 A.M.	5.5	9.0
		Corolla	7/11/23, 8:30 A.M.	14.5	15.5
			7/11/23, 8:30 A.M.	15.5	15.5

†† The outer florets were taken.

Larger differences were found in *Sparganium minimum*, in which the roots measured 2.5 atm. and the filaments 6.0 atm. In the two submersed aquatic plants in which I examined the suction force of the leaves and of the root tips simultaneously, that of the leaves was higher; e.g., the root tips of *Potamogeton crispus* had a suction force of 2.5 atm. while the leaf had 4.5 atm. Comparing the corolla with the lower foliar epidermis at the base of the midvein, I found the higher value in the former; e.g., in *Hieracium murorum* the values were 8.0 atm. and 5.5 atm., respectively.

It is particularly striking that in submersed aquatic plants suction forces of several atmospheres were found. In a number of plants which had none of their organs exposed to the air, these maximal values were determined:

<i>Potamogeton crispus</i> ,	leaves 6.0 atm., root tips 4.0 atm.
<i>Elodea canadensis</i> ,	leaves 4.5 atm., root tips 4.0 atm.
<i>Ranunculus flaccidus</i> ,	root tips 4.0 atm.
<i>Myriophyllum verticillatum</i> ,	leaves 7.5 atm., root tips 5.5 atm.

Since, as has been proved by experiments of Ursprung and Blum (19), the suction force of a cell lying in water soon falls to zero, it was naturally concluded that the submersed organs of aquatic plants should possess a zero suction force. This seemed the more plausible, since the simplified method gave results somewhat similar to those found by Ursprung and Blum. Thus the corolla of *Convolvulus sepium* which lay 9 hours in water showed a suction force certainly lower than 1.0 atm., and probably equal to zero. Nevertheless, our measurements show that the suction force of organs of submersed aquatic plants is not as low as zero. Why this is the case must be shown by further experiments. It might be mentioned in passing that this condition may be in part due to the processes of the ascent of sap, growth, assimilation, and other factors influencing osmotic value.

It should also be noted in this connection that in a young potted culture of *Zea Mays*, which clearly showed guttation, I found a suction force of 5.6 atm. in the leaf, and one of 4.8 atm. in the root tips; at the same time, in another plant which showed no guttation, I found a suction force of 5.6 atm. in the leaves and one of 4.4 atm. in the root tips.

Hardly anything is known of the changes in suction force in organs which are dying. Hence it might be of value to note the behavior of a corolla of *Convolvulus sepium* which was cut off and placed on my table in the laboratory. Fresh, it measured 4.0 atm.; after one hour the suction force had risen to 5.5 atm.; after two hours, contrary to expectation, it fell to 3.5 atm. This last measurement probably indicates one of the early stages of the dying process.

It would have been interesting to compare the suction force of a parasite with that of its host; unfortunately I had no opportunity of making such measurements. However, I measured a saprophyte, *Neottia Nidus-avis*, in which I found the fairly low values of 4.5 atm. in the perigone, and

3.5 to 4.0 atm. in the root tips. This is the more striking, when we consider that the plant grew upon a steep slope where the soil would dry out readily.

Of uncommon interest is the case in which two plants growing in the same humid forest, next to one another, showed large differences in their suction forces, although they were of the same height and systematically closely related. Thus a perigone of *Cephalanthera rubra* showed a suction force of 9.0 atm., while that of a neighboring *Plantanthera bifolia* showed one of 6.0 atm.

TABLE 54

Name of Plant	No. of Measurements	Maximum		Minimum		Amplitude	
		Suction Force in Atm.	Corresponding Bellis Values in Atm.	Suction Force in Atm.	Corresponding Bellis Values in Atm.	Suction Force in Atm.	Corresponding Bellis Values in Atm.
<i>Saponaria officinalis</i> .....	2	12.5	13.0	6.5	8.5	6.0	4.5
<i>Stellaria aquatica</i> .....	2	17.0	13.0	6.5	5.5	10.5	7.5
<i>Nymphaea alba</i> .....	2	4.5	9.5	4.5	13.0	0.0	3.5
<i>Helleborus foetidus</i> .....	5	66.0*		10.5	6.0	55.5	
<i>Aquilegia vulgaris</i> .....	2	7.5	7.0	6.5	7.0	1.0	0.0
<i>Chelidonium majus</i> .....	3	9.5	23.5	4.5	7.5	5.0	16.0
<i>Erucastrum nasturtiifolium</i> ...	2	15.5	6.0	9.5	8.5	6.0	2.5
<i>Saxifraga aizoides</i> .....	2	12.0	12.5	8.0	8.0	4.0	4.5
<i>Comarum palustre</i> .....	2	9.5	6.5	7.5	8.5	2.0	2.0
<i>Potentilla anserina</i> .....	6	12.0	7.5	6.0	7.0	6.0	0.5
<i>Geum urbanum</i> .....	2	10.5	7.0	7.5	8.5	3.0	1.5
<i>Rosa arvensis</i> .....	2	10.5	6.0	9.5	6.0	1.0	0.0
<i>Melilotus albus</i> .....	4	9.5	8.0	6.0	7.0	3.5	1.0
<i>Trifolium medium</i> .....	5	12.5	9.5	10.5	8.0	2.0	1.5
<i>T. pratense</i> .....	43	12.0	13.0	5.5	9.0	6.5	4.0
<i>T. repens</i> .....	2	9.5	10.0	7.5	10.0	2.0	0.0
<i>Geranium Robertianum</i> .....	11	9.0	11.0	2.5	9.5	6.5	1.5
<i>Hypericum perforatum</i> .....	2	9.5	10.5	9.5	8.0	1.0	2.5
<i>Epilobium angustifolium</i> .....	2	8.0	17.5	6.0	13.5	2.0	4.0
<i>E. montanum</i> .....	5	9.0	5.5	6.5	8.0	2.5	2.5
<i>Lysimachia nemorum</i> .....	3	6.0	10.5	6.0	10.5	0.0	0.0
<i>Convolvulus sepium</i> .....	4	14.5	8.0	3.5	11.0	11.0	3.0
<i>Echium vulgare</i> .....	2	9.0	8.0	6.5	21.0	2.5	13.0
<i>Satureia vulgaris</i> .....	3	15.5	21.5	7.5	6.0	8.0	15.5
<i>Solanum nigrum</i> .....	5	8.0	9.0	5.5	9.5	2.5	0.5
<i>Linaria vulgaris</i> .....	2	23.5	21.0	8.0	5.5	15.5	15.5
<i>Linaria minor</i> .....	2	9.5	10.0	8.0	7.0	1.5	3.0
<i>Asperula odorata</i> .....	2	5.5	8.5	5.5	7.5	0.0	1.0
<i>Scabiosa columbaria</i> .....	2	21.5	12.0	5.5	5.5	16.0	6.5
<i>Campanula rotundifolia</i> .....	3	15.5	8.0	8.0	11.0	7.5	3.0
<i>Campanula Rapunculus</i> .....	2	13.5	16.0	6.0	7.0	7.5	9.0
<i>Bellis perennis</i> .....	2	11.0	10.0	5.5	5.5	5.5	4.5
<i>Tussilago Farfara</i> .....	5	23.0*		14.5*		8.5	
<i>Chrysanthemum Leucanthemum</i>	3	16.0	15.5	4.5	8.5	11.5	7.0
<i>Cirsium arvense</i> .....	2	11.0	10.0	11.0	7.0	0.0	4.0
<i>Cichorium Intybus</i> .....	2	12.0	16.0	9.5	19.5	2.5	3.5
<i>Cicerbita muralis</i> .....	2	9.5	18.0	7.5	16.0	2.0	2.0
<i>Sonchus oleraceus</i> .....	2	12.0	7.0	6.5	10.0	5.5	3.0
<i>Hieracium murorum</i> subsp.							
<i>circumstellatum</i> .....	2	8.0	9.0	6.5	7.5	1.5	1.5
<i>Hieracium murorum</i> subsp.							
<i>gentile</i> .....	2	15.5	15.5	14.5	15.5	1.0	0.0

\* Winter measurements.

In closing, let us, by means of table 54, attempt to draw some conclusions concerning the behavior of different species and families. That families composed only of submersed aquatic plants have relatively low suction-force values need not be insisted upon. The same holds for families which thrive only in fairly moist places. It is difficult to make comparisons between the suction forces of different species because of diurnal and annual periodicity. A perfect comparison would be possible only if we had the complete diurnal and annual suction-force curves of each species for all the habitats in which they occur. At present we are still far from having realized this ideal. Still, from the material at hand, it is evident that various plants behave very differently.

Table 54 gives a slight, although extremely imperfect, insight into this condition. It shows the maxima and minima, as well as the amplitude of suction-force variations, of the corollas of all species measured more than once. In order that these measurements may be better compared, the corresponding simultaneously measured Bellis values are also given.

The highest maximal values, if winter measurements are left out of consideration, are found in the following species. (The figures in parentheses are those of the corresponding Bellis values.)

<i>Linaria vulgaris</i> .....	23.5 atm. (21.0 atm.)
<i>Scabiosa columbaria</i> .....	21.5 atm. (12.0 atm.)
<i>Stellaria aquatica</i> .....	17.0 atm. (13.0 atm.)
<i>Chrysanthemum Leucanthemum</i> .....	16.0 atm. (15.5 atm.)
<i>Campanula rotundifolia</i> .....	15.5 atm. ( 8.0 atm.)
<i>Satureia vulgaris</i> .....	15.5 atm. (21.5 atm.)
<i>Erucastum nasturtiiifolium</i> .....	15.5 atm. ( 6.0 atm.)
<i>Hieracium murorum</i> .....	15.5 atm. (15.5 atm.)

The Bellis values show that the comparatively highest suction force is not that of *Linaria vulgaris* but that of *Scabiosa columbaria*. Again we see that the absolute value of 15.5 atm., in reality, represents entirely different relative values; since the corresponding value of Bellis for *Erucastum* is less than one half as great, and for *Satureia* roundly one and one third times as great.

Practically all the plants with high suction-force maxima grew in dry places. That fairly high suction forces can be developed in places which are not so dry is shown by *Satureia*. That plants growing in dry places may retain low suction forces is shown most clearly by *Geranium Robertianum*, which was repeatedly taken for measurement from a dry wall and which always showed notably lower values than the control Bellis. Most characteristic is the measurement of July 13, 1923, when a *Geranium* value of 6.5 atm. from a dry wall corresponded with a Bellis suction force of 23.5 atm. In agreement with this observation, we also find for *Geranium Robertianum* a minimum of 2.5 atm. By examination of the amplitude column, it may be seen how differently various species behave.

## SUMMARY

1. The introduction gives a short *résumé* of studies made, in recent years, on suction force and turgor pressure.

2. My own study is limited to work with suction force by means of the newly devised "simplified method," which does not permit the measurement of individual cells, but instead that of strips of tissues.

3. Soil moisture and humidity of the air are the external factors which especially influence suction force. For instance, a strong rain after a drought may cause the suction force of leaves to drop considerably (more than 20 atm.). In winter, temperature also plays an important rôle for land plants.

4. The suction forces of land plants, which alone had previously been examined with a view to periodicity, very clearly show periodic variations. The diurnal suction-force curve, with a maximum toward noon, must be ascribed principally to variations in atmospheric moisture. The annual curve depends primarily upon precipitation, and in winter also upon low temperatures.

5. The effect produced by various habitats upon the suction force is evident. The lowest values are found in submersed aquatic plants, but these are not as low as one might expect; with increasing dryness of habitat, suction force increases.

6. Different species may show quite different suction-force values, which depend partly upon the habitat and partly upon the characteristics of a given species.

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## CORRECTION

In part I of this paper, on page 458, the reference in the first line below table 33 should be to page 445 instead of 439.



# FACULTATIVE PARASITISM AND HOST RANGES OF FUNGI<sup>1</sup>

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## INTRODUCTION

Study of the supposedly large group of saprophytic fungi shows that it is surprisingly small. Increasing evidence shows that many fungi, formerly called saprophytes, are able to enter living plants and cause diseased conditions in one or more cells. It is worth while to study facultative parasites, not only because of the damage that they have done already, but also because of the danger that they may become more virulent. There are many cases of diseases, formerly unknown or of little importance, which have become serious and widespread. Some examples of these are: apple blotch (*Phyllosticta solitaria*), peach black spot (*Pseudomonas pruni*), corn black-bundle disease (*Cephalosporium acremonium*), and Panax leaf spot and carrot rot caused by species of *Alternaria*. Change in virulence is noted in the statement by Whetzel (61) that, in Bermuda, *Alternaria solani* in potatoes produced symptoms resembling late blight.

Arzberger (2) says that *Coniosporium gecevi* is an obligate saprophyte which causes damage by rotting corn cobs; this is comparable to the way in which some species of *Armillaria*, *Marasmius*, *Polyporus*, *Stereum*, and *Lycoperdon gemmatum* Bat. cause the damage that gives some basis for calling them parasites.

For the purpose of considering the cross inoculations, a fungus was tentatively said to be the cause of a leaf or stem spot when diseased lesions appeared below the spores or mycelium used in inoculation and not elsewhere within 1-7 (usually 2-4) days after inoculation; many isolations and repeated inoculations were made. The term "new diseases" is used here tentatively to indicate previously undescribed diseases which resulted when single species of plants were inoculated with pure cultures of fungi; aseptic plants were used in the large tubes.

The data given here are intended to show how many "new diseases" resulted from the cross inoculations, to illustrate the facultative parasitism of *Alternaria*, *Macrosporium*, and *Helminthosporium*, and to give informa-

<sup>1</sup> Penetration phenomena exhibited by the inoculated plants are described in a recently published paper. (Young, P. A. Penetration Phenomena and Facultative Parasitism in *Alternaria*, *Diplodia*, and other fungi. Bot. Gaz. 81: 258-278. 1926.) The author wishes to thank Dr. F. L. Stevens, professor of plant pathology at the University of Illinois, for numerous helpful suggestions, Mr. H. E. Morris, associate botanist and bacteriologist at the Montana Agricultural Experiment Station, for criticizing the manuscript, and all others who have given aid.

tion concerning the experimental host ranges of many fungi. This work represents only a beginning in the study of facultative parasitism.

#### REVIEW OF LITERATURE

Ward (59) gives a general discussion of parasitism. Very significant is the statement by Brierley (6) that the researches of Massee (32) did not justify the conclusion that, by injecting chemicals into prospective host plants, saprophytes can be induced to enter them and parasites can be induced to attack new hosts. Welles (60) states that species of *Cercospora* are very indiscriminate with regard to hosts attacked. Rosen and Elliott (43) say that infection of wheat by *Ophiobolus* appears to be confined to weakened plants.

Tisdale (55) pictures cuticular penetration by *Alternaria*. Sorauer (48) and Jones (26) report stomatal penetration by *Alternaria solani*; the latter says that cuticular penetration also occurs. Rands (39) says that *Alternaria crassa* caused incipient infections of potato, tomato, and *Solanum nigrum* leaves in the form of tiny brown specks; these spots did not enlarge. Drechsler (14) says that discoloration of wheat grains by *Alternaria* is less intense than that caused by *Helminthosporium*. Stakman (50) says that *Alternaria*, *Botrytis*, *Colletotrichum phomoides*, *Gliocladium*, *Tilachlidium*, and *Trichoderma* were parasitic on cereal roots. Elliott (19) made cross inoculations with *Alternaria*.

Bolley (5) says that *Alternaria*, *Macrosporium*, and *Helminthosporium* blight wheat ovules and attack other cereals. Meier, Drechsler, and Eddy (33) describe *Alternaria radicina* as a serious facultative parasite. Cook and Martin (10), Longyear (28), and Roberts (41) describe some species of *Alternaria* as parasites on apple. Faulwetter (20) says that *Alternaria tenuis* is a weak parasite of cotton leaves. Gibson (21) says that *Alternaria atrans* is weakly parasitic on soybean and cowpea leaves.

In inoculation tests, Edson and Shapovalov (18) found that *Alternaria* caused brown cankers on subterranean potato stems. Chen (8) found *Alternaria* in cabbage and bean seeds and *Macrosporium* in bean and cowpea seeds; he says that *Rhizopus nigricans* killed tomato seedlings in tubes but not in the greenhouse. Beckwith (4) found *Macrosporium* and *Helminthosporium* in surface-sterilized wheat stems. Crawford (12) isolated *Alternaria* and *Cephalothecium* from the interior of cotton seeds. Out of 3,203 trials, Edson (17) isolated *Alternaria* from potato tubers 615 times and *Penicillium* 104 times.

Higgins (24) reports cuticular penetration of pepper fruits by *Macrosporium solani*. Teodoro (53) says that *Macrosporium parasiticum* is parasitic on onions. Rosenbaum and Sando (44) found that the mechanical pressure needed to puncture tomato fruits increased with the age of the fruits, and that the percentages of infections with *Macrosporium tomato* decreased with the age of the fruits. Brisley (7) says that *Macrosporium*

*ucumerinum* caused weak infections of potato and tomato and did not infect cabbage, turnip, bean, and orange.

Stevens (51), Christensen (9), and Stakman (49) present evidence of the wide host ranges of species of *Helminthosporium*. Stevens (51), Mcfemia (38), Nisikado and Miyake (35), and Noack (36) describe the penetration phenomena of *Helminthosporium*. Stover (52) says that *Helminthosporium* causes a seedling blight of corn.

Shapovalov (45) inoculated uninjured potatoes with *Penicillium oxalicum* and found that it caused dark, depressed lesions. He says such potential parasites deserve attention because of the possibility of their becoming progressively more strongly parasitic. Hurd (25) says that *Penicillium* injures wheat in the soil and in the elevator. Durrell (15) found that *Fusarium*, *Penicillium*, *Rhizopus*, and two species of bacteria caused purple sheath spots of corn when inoculated with media. Manns and Adams (31) report *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Helminthosporium*, *Rhizopus*, *Spicaria*, *Hormodendrum*, *Torula*, *Chaetomium*, *Colletotrichum*, and several species of bacteria in corn. Nordhausen (37) placed *Penicillium* in a wound in a leaf and found that it grew well, but did not invade the uninjured cells; he placed spores and gelatine on leaves and found that the mold did not injure the leaves.

Miyoshi (34) pictures *Botrytis* penetrating a cellulose wall impregnated with paraffin and states that *Botrytis* and *Penicillium* bored through gold leaf. Heald and Dana (23) say that *Botrytis* is a weak wound parasite of apple fruits. Anderson (1) says that no species of flowering plants is immune from the ravages of *Botrytis cinerea*, especially under greenhouse conditions, but that he has not seen it directly infecting vigorous, green leaves. Wollenweber (62) says that *Ramularia* occurs in tuber and fruit rots and on dung in the soil. Cross inoculations with a wheat *Fusarium* by MacInnes and Fogelman (29) resulted in infections of many unrelated hosts. Rathburn-Gravatt (39 a) inoculated coniferous seedlings with many fungi.

Barrus (3) and Leach (27) describe the formation of minute, incipient infection spots in resistant beans by *Colletotrichum*. Shear and Wood (46) say that haustoria from appressoria of *Glomerella* may penetrate a very short distance and stop temporarily; they made many cross inoculations. Halsted (22), Edgerton (16), Sheldon (47), and Cook and Taubenhause (11) report many cross inoculations with anthracnose fungi; Barrus (3), Leach (27), Shear and Wood (46), Walker (57), and Dey (13) describe penetration by such fungi.

#### METHODS

Fungi were grown on cornmeal agar. Agar was poured directly from flasks into Petri dishes. Seeds were surface-sterilized by soaking for 10-30 minutes in a 3 percent solution of Chloramine-T or in a 0.5-1 percent solution of Uspulun. Many samples were presoaked for 1-5 hours. Uspu-

lun was generally 100 percent effective while Chloramine-T was only 95-100 percent effective in giving aseptic seedlings. Uspulun reduced the percentage of germination of wheat 5-15 percent; Chloramine-T reduced it 15-25 percent.

Seeds were transferred from the disinfectants, without washing, to the wet filter paper in the bottoms of large autoclaved dishes of the Petri-dish type; the dishes were then kept in a dark incubator at 25° C. Usually within 3-5 days, the plumules of cereal seedlings reached a length of 1-3 cm. without rupturing the coleoptiles.

Strips of cloth 15-25 by 5 cm. were wet with tap water, rolled up, and placed in tubes of the test-tube type which were 18-25 by 2 cm.; tubes were stoppered with cotton and autoclaved. Using aseptic technique, a rag was removed from a tube, unrolled with forceps in the bottom of a sterile dish, and usually 6 seedlings were placed on it; seedlings were not mechanically injured. This method was used by Stevens (51).

Fungous spores or pieces of agar bearing mycelium were placed on the plumules, but not in contact with the seeds. After inoculation, the seedlings were rolled in the rag and placed in the tube; wet cloth was in contact with the inoculated regions. Seedlings were never, and the rags rarely, touched with the hands during the inoculation process. Seedlings were grown in the light at 20-28° C. Check seedlings rarely revealed the presence of fungi or bacteria during the first week after being placed in tubes; they often remained aseptic longer than one week. Usually within 3-7 days after inoculation, strips of the outer layers of cells of inoculated regions were removed from the plumules, stained, and examined.

Apples were washed with a 0.2 percent  $\text{HgCl}_2$  (or 0.5-1 percent Uspulun) solution. Four "V"-shaped wounds were made in each apple, the tips of the incisions were raised, agar bearing mycelium was inserted into the cavities, the tips were pressed back into place, and the apples were stored in sterile dishes with wet filter-paper linings.

The outer scales were removed from onion bulbs which were then surface-sterilized like the apples, almost vertically severed, some of the thin membranes between the scales inoculated, a tight band being added to hold the scales in a normal position, and the bulbs were then stored like the apples. Such onions produced roots and leaves and remained in a fairly good condition for a week.

In the greenhouse, seeds without surface-sterilization were planted in unsterilized soil. Aseptic technique was used in transferring fungi from agar plates to the leaves. Inoculations were made on apparently normal leaves which were not mechanically injured; the fungi were placed in drops of water on the leaves. Spore inoculations were covered with cover glasses; this often arranged the floating spores in rings marking the boundaries of the flattened drops of water. When necessary, as with grass, cabbage, and some other plants, small regions of leaves were quickly

washed with 95 percent ethyl alcohol in cotton so that drops of water would adhere to them. Inoculation by placing mycelium growing on agar in contact with leaves was often more effective than spore inoculations.

Large glass or crockery jars (hereafter called bell jars) were lined with 1 or 2 layers of filter paper wet, at least the first time, with a 0.2 percent  $\text{HgCl}_2$  solution. The jars were covered with burlap sacks to exclude sunlight and to reduce the injury to the enclosed plants. Inoculated plants were covered with these jars and sacks for usually 3 days, after which time the interiors of the bell jars were dried slowly by raising the edges 2-5 cm. above the soil and leaving the plants covered for 1-2 days.

Potted plants were placed in large glass cases, inoculated, and water was constantly sprayed into the cases for 3 days. Also, large plants growing in the greenhouse beds were inoculated and water was sprayed on to them. Such inoculations usually did not result in infections.

Accurate classification of most species of *Alternaria* and *Macrosporium* does not appear to be possible; Elliott (19) and Roberts (42) have pointed out some of the difficulties met in classifying them. Sizes of *Alternaria* spores varied widely, and the ones formed in culture were often different from those on the plants from which the cultures were isolated. Cultures were considered to be distinct species or physiological forms when they differed in origin, cultural characters, or parasitism.

The specific names given to dematiaceous fungi are probably correct; they were given the names of the described species with which they agreed most closely in sizes of spores and original hosts. No two cultures of *Alternaria* or *Macrosporium* appeared to be morphologically and physiologically identical; the physiological forms of *Alternaria tenuis* Nees were clearly distinct. A tabulation of the genera *Alternaria* and *Macrosporium* was used. The names which fungi bore when received were generally given to fungi received from other persons.

Greenhouse plants were said to be infected when prominent leaf spots appeared at the points of inoculation and not elsewhere within a few days. In the tube inoculations, plants were said to be infected when microscopical examination showed internal mycelium, large regions of browned host cells associated with superficial mycelium, or callosities. In the tubes in which infections occurred, most of the seedlings were infected; the more strongly parasitic fungi usually infected all the seedlings in the tubes.

In a previous article, the name "callosity" (Pl. XXXVII, figs. 1, 7) was proposed for the usually globular or cylindrical, hyaline to brown bodies formed by wheat and many other hosts on and in their cell walls when stimulated by infection by *Alternaria*, *Macrosporium*, *Helminthosporium*, *Acrothecium*, *Diplodia*, *Colletotrichum*, *Cephalosporium*, and probably some other fungi. Their formation seems to be a typical reaction by some hosts to infection by these fungi. Penetration hyphae are usually enclosed by or attached to callosities (Pl. XXXVII, figs. 1, 7). Under

the tentative name "calluses," Stevens (51) discussed the bodies here called callosities.

#### CROSS INOCULATIONS

Unless otherwise stated, "wheat" means Red Wave wheat. *Impatiens pallida* Nutt. was used in many experiments; the generic name is used alone hereafter. Authorities for botanical names are given the first time each name is used; common names are often given with the binomial names and are used alone thereafter. An asterisk (\*) after a host name means that a "new disease" occurred on this plant. Only seedlings were inoculated in tubes, and only leaves were inoculated in the greenhouse. The following records were secured usually within 1-7 (often 2-4) days after inoculation. These data were condensed from 69 tables.

#### *Alternaria*

*A. brassicae* (Berk.) Sacc. f. *microspora* P. Brun. P.F. 1<sup>2</sup> from cabbage leaf spot. Besides those shown in Plate XXXVI, D, the following results from greenhouse inoculations were secured: Early Buff cowpea (*Vigna sinensis* Endl.), 0/28<sup>3</sup>; Premium Gem pea (*Pisum sativum* L.), 5/29; purple lilac (*Syringa vulgaris* L.), 0/9; onion bulb (*Allium cepa* L.), 0/4; *Sonchus oleraceus* L., 0/27; *Acer negundo* L., 0/11; *Portulaca oleracea* L., 0/12; *Capsella bursa-pastoris* (L.) Medic., 0/15; and *Chenopodium album* L., 0/10. Wounded apple fruit (*Pyrus malus* L.) and unwounded fruit of *Berberis thunbergii* DC. were not infected. Barley (*Hordeum vulgare* L.) seemed to be immune. Green leaf spots appeared in yellow radish leaves (*Raphanus sativus* L., Pl. XXXVII, fig. 5). Only wound inoculations were successful in bean leaves (*Phaseolus* sp.). "New diseases" occurred in White Italian broom corn (*Holcus sorghum* L. var. *technicus* Bailey), pea, wax bean, radish, and muskmelon (*Cucumis melo* L.).

*A. brassicae* (Berk.) Sacc. f. *microspora* P. Brun. P.F. 2 from cabbage leaf spot. In tubes, most of the 30 inoculated wheat\* seedlings (*Triticum aestivum* L.) were infected. Some of the 60-day oats\* (*Avena sativa* L.), Amber Cane sorghum\* (*Holcus sorghum* L. var. *saccharatus* Bailey), cabbage\* (*Brassica oleracea* L.), turnip\* (*Brassica rapa* L.), and tomato\* (*Lycopersicon esculentum* Mill.) seedlings were infected; pumpkin seedlings (*Cucurbita pepo* L.) were not infected. P.F. 1 infected old cabbage leaves in the greenhouse while P.F. 2 did not.

*A. ribis* Bubak & Ranojevic from *Ribes grossularia* L. leaf spot. Plate XXXVI, A gives the cross inoculations. Infection phenomena are shown in Plate XXXVII, fig. 1. "New diseases" occurred in wheat, barley, oats, sorghum, pop corn (*Zea mays* L. var. *evarta* Bailey), soybean (*Glycine max*

<sup>2</sup> "P.F." means physiological form as defined in Phytopathology 15: 316-317. 1925.

<sup>3</sup> In the fractions accompanying the names of flowering plants, the denominators refer to the numbers of inoculations and the numerators refer to the numbers of infections (leaf or stem spots).

Merr.), cabbage, radish, turnip, tomato, watermelon (*Citrullus vulgaris* Schrad.), Impatiens, and *Ribes odoratum* Wendl.

*A. makhæ* Roum. & Let. from *Abutilon theophrasti* Medic. leaf spot. In tubes, it infected most of the wheat\* and sorghum\* and some of the pop corn\*, cabbage\*, radish\*, turnip\*, tomato\*, and pumpkin\* seedlings. It did not infect onion bulb membranes or leaves.

*A. rugosa* McAlp. P.F. 1 from tomato end rot; it resembles *Macrosporium lycopersici* Plowr. and *M. tomato* Cooke. Besides those shown in Plate XXXVI, B, the following results from greenhouse inoculations were secured: egg plant (*Solanum melongena* L.), 0/10; wax bean, 0/12; *Tetragonia expansa* Murr., 0/15; petals of red tulip (*Tulipa gesneriana* L.), 0/11; *Commelina communis*? L., 0/6; Impatiens\*, 12/12; *Datura tatula* L., 0/28; *Solanum nigrum*\* L., 4/5; *Chenopodium album*, 0/7; *Saponaria officinalis* L., 0/12; *Acer negundo*, 0/10; and onion leaves, 0/4. Red infection rings appeared under the rings of spores on sorghum leaves. Other "new diseases" occurred in wheat, barley, sorghum, pop corn, pumpkin, soybean, cabbage, radish, turnip, tomato, spinach (*Spinacia oleracea* L.), Swiss chard (*Beta vulgaris* L. var. *cicla* L.), and pea.

*A. rugosa* McAlp. P.F. 2 from pepper (*Capsicum frutescens* L. var. *grossum* Bailey) end rot. It infected wheat\* in a tube. Results of greenhouse inoculations: pepper\*, 5/6; sorghum\*, 5/10; radish\*, 8/8; spinach, 0/13; Impatiens\*, 11/11; *Datura tatula*\*, 20/31; *Solanum nigrum*\*, 15/15; *Podophyllum peltatum* L., 0/11; and *Acer negundo*, 0/18.

*A. rugosa* McAlp. P.F. 3 from eggplant fruit rot. It infected wheat\* and tomato\* seedlings in tubes.

*A. tenuis* Nees P.F. 1 from wheat seed. Results of greenhouse inoculations: sorghum 3/3; broom corn\*, 8/10; wheat, 0/25; oats, 10/10; Yellow Dent corn\* (*Zea mays* L. var. *indentata* Bailey), 14/14; pop corn 3/14; spinach, 0/12; *Hemerocallis fulva* L., 0/10; Impatiens, 6/6; radish\*, 3/8; and *Ribes odoratum*\*, 5/7. Red infection rings appeared below the rings of spores on sorghum leaves. Two green leaf spots appeared in yellow radish leaves.

*A. tenuis* Nees P.F. 2 from wheat seeds. In tubes, it infected most of the 40 Red Wave and the 6 Illinois No. 1 wheat\* seedlings. It also infected barley\*, oats\*, pop corn\*, sorghum\*, pumpkin\*, cabbage\*, tomato\*, and *Abutilon theophrasti*\*. Onion bulbs and apples were not infected. In the greenhouse, cabbage, onion, and wheat leaves were not infected; 17 inoculations of Impatiens\* leaves resulted in some leaf spots.

*A. tenuis* Nees P.F. 3 from wheat seedlings. It infected wheat, tomato, and turnip\* seedlings in tubes.

*A. tenuis* Nees P.F. 4 from leaf mold of *Kentia sanderiana*?; spores in culture were too small to agree with the description of *A. pulvinata* Cooke and Mass. It infected wheat\* and sorghum\* in tubes. Results of greenhouse inoculations: pop corn\*, 14/14; wheat, 0/7; and sorghum 2/17.

*A. tenuis* Nees P.F. 5 from millet infected Red Wave and Illinois No. 1 wheat\* in tubes.

*A. tenuis* Nees P.F. 6 from radish leaf spot; it resembles *Macrosporium brassicae* Berk. and *M. commune* Rabh. In tubes, it infected wheat\*, oats\*, Rosen rye\* (*Secale cereale* L.), sorghum\*, cabbage\*, radish\*, and pumpkin\*. Results of greenhouse inoculations: cabbage, 0/30; radish, 14/24; wheat, 0/9; sorghum, 5/8; broom corn\*, 2/3; barley\*, 8/9; corn\*, 5/12; cowpea\*, 6/11; *Commelina communis*?, 0/5; Impatiens\*, 5/5; and sugar beet\* (*Beta vulgaris* L.), 3/5.

*A. tenuis* Nees P.F. 7 from *Datura tatula* leaf spot; spores too small to agree with the description of *A. crassa* (Sacc.) Rands. In tubes, it infected most of the 24 Red Wave and the 6 Illinois No. 1 wheat\* seedlings; also some of the sorghum\*, pop corn\*, and cabbage\*. Pumpkin seedlings and onion bulb membranes were not infected. In the greenhouse, the 4 inoculations on potato (*Solanum tuberosum* L.) and the 16 on cabbage did not cause leaf spots.

*A. tenuis* Nees P.F. 8 from bean pod mold. In tubes, it infected wheat\*, soybean\*, and cabbage\*. It did not infect wax-bean leaves in the greenhouse.

*A. tenuis* Nees P.F. 9 from fruit rot of *Symphoricarpos albus* Blake infected wheat\* and oats\* in tubes. It did not infect cabbage leaves in the greenhouse.

*A. tenuis* Nees P.F. 10 from moldy tops of *Asparagus officinalis* L. var. *altilis* L. It infected wheat\* in tubes but did not infect *Hemerocallis fulva* leaves in the greenhouse.

*A. tenuis* Nees P.F. 11 from rose-bud mold. In tubes, it infected wheat\*, broom corn\*, muskmelon\*, and pumpkin\*. It caused 14 corn\* leaf spots in the greenhouse.

*A. tenuis* Nees P.F. 12 from privet (*Ligustrum*) leaf spot infected wheat\* and sorghum\* in tubes; 11 inoculations on privet leaves in the greenhouse failed to cause leaf spots.

*A. tenuis* Nees P.F. 13 from lilac (*Syringa vulgaris*) leaf spot infected wheat\* in tubes and did not infect lilac leaves in the greenhouse.

*A. tenuis* Nees P.F. 14 from apple-core mold. In tubes, it infected wheat\* and sorghum\* and did not infect cabbage and turnip. It did not rot an apple.

*A. tenuis* Nees P.F. 15 from tomato leaf spot; spores in culture were much too small to agree with the description of *A. solani* (E. & M.) J. & G. It infected wheat\* and tomato\* seedlings in tubes.

*A. tenuis* Nees P.F. 16 from *Viburnum opulus* L. leaf spot infected wheat\* and cabbage\* in tubes; no internal hyphae were seen.

*A. brassicae* (Berk.) Sacc. f. *phaseoli* P. Brun. from a cowpea leaf spot; it resembles *Macrosporium phaseoli* Faut. and *M. fasciculatum* C. & E. It infected wheat\* in tubes. In the greenhouse, 15 inoculations on barley and 17 on *Hemerocallis fulva* did not cause leaf spots.



*Alternaria* sp. from oat leaf mold; it resembles *Macrosporium avenae* Oud. The spores in culture were catenulate. The cross inoculations are shown in Plate XXXVI, C. "New diseases" occurred in wheat, sorghum, pop corn, corn, cabbage, radish, and *Melilotus alba* Desr. Onion leaves were not infected. An auto-stained red infection ring is shown under a ring of spores in Plate XXXVII, figure 6.

### Macrosporium

*M. iridis* C. & E. from Iris leaf spot infected wheat\*, broom corn\*, corn\*, muskmelon\*, and pumpkin\* seedlings in tubes. It infected onion\* bulb membranes. Results of greenhouse inoculations: corn, 5/37; sorghum, 0/14; pea\*, 3/18; *Chenopodium album*\*, 3/11; cabbage\*, 10/10; *Hemerocallis fulva*, 0/16; and *Tragopogon porrifolius* L., 0/5.

*M. catalpae* Ell. & Mart. from Catalpa leaf spot infected pumpkin\*, wheat\*, and broom corn\* in tubes. Results of greenhouse inoculations: corn\*, 5/14; broom corn, 0/10; barley, 0/11; and icicle radish\*, 6/8.

*M. diversisporum* Thüm. P.F. 1 from corn leaves; it resembles *M. maydis* C. & E. In tubes, it infected wheat\* and sorghum\*. Results of greenhouse inoculations: corn, 5/8; pop corn\*, 5/10; oats\*, 5/6; sorghum, 2/20; Impatiens\*, 5/5; and radish\*, 4/9.

*M. diversisporum* Thüm. P.F. 2 from corn husks infected wheat in a tube. Results of greenhouse inoculations: sorghum, 9/9; broom corn, 0/16; corn, 9/26; wheat, 0/26; pop corn, 11/17; blue violet (*Viola*), 0/12; spinach, 0/14; *Acer negundo*, 0/14; dandelion (*Taraxacum officinale* Weber), 0/14; *Smilacina racemosa* (L.) Desf., 0/13; Impatiens, 10/10; grapefruit (*Citrus maxima* Merr.), 0/3; and lilac, 0/9.

*M. parasiticum* Thüm. (first isolation) from onion leaf spot; it resembles *M. caepicola* Speg. and *M. alliorum* Cooke & Mass. It infected wheat\* in a tube. It infected onion\* bulb membranes, but did not infect onion leaves.

*M. parasiticum* Thüm. (second isolation) from onion leaf spot infected wheat in a tube and directly penetrated the cells of an onion bulb membrane in a dish.

*M. cookei* Sacc. from pepper (*Capsicum*) leaf spot; it resembles *M. commune* Rabh. and *Alternaria tenuis* Nees. It infected wheat\*, sorghum\*, pop corn\*, cabbage\*, radish\*, and *Abutilon theophrasti*\* in tubes. Ten inoculations of *Hemerocallis fulva* leaves in the greenhouse failed; onion bulb membranes were not infected.

*M. saponariae* Peck from *Saponaria officinalis* L. leaf spot infected wheat\* and cabbage\* in tubes. The 11 inoculations of *Saponaria officinalis* in the greenhouse caused no leaf spots.\*

*M. ignobile* Karst. from rye leaf spot infected wheat\* and pop corn\* in tubes.

*M. nitens* (Fres.) Sacc. P.F. 1 from *Cucurbita maxima* Duchesne fruit

rot; it resembles *M. lagenariae* Thüm. and *M. granulosum* Bubak. It infected wheat\*, sorghum\*, pop corn\*, and tomato\* seedlings in tubes, but not an onion bulb in a dish. Results of greenhouse inoculations: muskmelon\*, 4/12; Swiss chard\* seedlings, 6/7; tomato, 2/3; sorghum, 0/12; and *Impatiens*\*, 1/10.

*M. nitens* (Fres.) Sacc. P.F. 2 from *Cucumis sativus* L. fruit rot infected wheat\* in tubes. Results of greenhouse inoculations: sorghum, 0/23; *Acer negundo*, 0/13; blue violet, 0/12; and *Tetragonia expansa*, 0/15.

*M. sydowianum* Farneti P.F. 1 from superficial apple rot infected wheat\* in a tube and failed to rot wounded apples in dishes. It caused *Impatiens*\* leaf spots in the greenhouse.

*M. sydowianum* Farneti P.F. 2 from fruit of *Cydonia oblonga* Mill. infected wheat\* in tubes and did not infect cabbage in the greenhouse.

*M. cucumerinum* E. & E., received from H. R. Brisley (7). It infected wheat\* in tubes. It did not rot an apple. Results of greenhouse inoculations: wheat, 0/13; corn\*, 15/22; *Hemerocallis fulva*, 0/5; and lilac, 0/6.

#### Helminthosporium and Acrothecium

*H. gramineum* (Rabh.) Erik. from wheat leaf spot infected wheat, corn\*, pumpkin\*, and muskmelon\* seedlings in tubes. Results of greenhouse inoculations: wheat, 12/12; oats\*, 8/10; sorghum\*, 23/39; rye\*, 14/14; corn, 13/32; barley\*, 21/25; cabbage\*, 2/37; cotton\* (*Gossypium* sp.), 8/19; *Commelina communis*?, 0/10; tomato, 0/10; potato, 0/5; *Datura tatula*\*, several leaf spots; onion leaves, 0/18; *Ricinus communis* L., 0/12; *Sonchus oleraceus*, 0/9; dandelion, 0/16; *Amaranthus* sp.\*, 5/17; *Chenopodium album*, 0/18; *Polygonum* sp., 0/9; apple leaves, 0/10; *Ribes odoratum*, 0/14; *Trillium recurvatum* Beck, 0/9; Swiss chard, 0/25; wax bean\*, 5/36; cowpea, 0/40; *Trifolium pratense* L., 0/3; pea, 0/5; *Acer negundo*, 0/13; *Ornithogalum umbellatum* L., 0/18; *Plantago rugelii* Dcne., 0/15; *Rumex crispus* L., 0/17; *Impatiens*\*, 11/11; lilac, 0/12; *Callistephus chinensis* Nees., 0/16; *Smilacina racemosa*, 0/9; celery (*Apium graveolens* L. var. *dulce* DC.), 0/9; *Vinca minor*, 0/30; muskmelon, 0/10; *Conwallaria majalis* L., 0/4; spinach, 0/7; grapefruit, 0/3; *Tetragonia expansa*, 0/11; *Lamium amplexicaule* L., 0/10; *Capsella bursa-pastoris* (L.) Medic., 0/17; and *Saponaria officinalis*, 0/12. Green spots appeared in wheat and rye leaves that had turned yellow under the bell jars (Pl. XXXVII, figs. 2-4).

*H. gramineum* (Rabh.) Erik. from a wheat seedling. Results of greenhouse inoculations: broom corn, 0/12; *Ribes odoratum*, 0/6; and *Melilotus alba*, 0/8.

*Helminthosporium* sp. Many 1- to 3-celled spores occurred in the cultures. It infected wheat\*, sorghum\*, broom corn\*, and pumpkin\* seedlings in tubes.

*Helminthosporium* sp. from barley leaf mold; many 1- to 3-celled spores occurred in the cultures. It infected wheat\*, muskmelon\*, and pumpkin\*

seedlings in tubes. Results of greenhouse inoculations: barley, 12/12; corn\*, 14/14; cowpea, 0/15; and pea\*, 3/15. New spores and sporophores appeared on the green leaf spots in green barley leaves within 3 days after inoculation.

*Acrothecium* sp. infected wheat\* in tubes and resembled a weak *Alternaria* in its parasitism. Results of greenhouse inoculations: barley, 0/12; sorghum, 0/16; and broom corn, 0/12. It did not infect cabbage leaves.

### Other Fungi

*Diplodia zeae* (Schw.) Lév. from corn kernels. Most of the 25 wheat\* coleoptiles inoculated in tubes became dark brown, formed innumerable callosities, and often exhibited superficial white mycelium (Pl. XXXVII, figs. 7-11). Some muskmelon\* seedlings in tubes were infected. Onion bulb membranes were not infected. Results of greenhouse inoculations: corn, 15/15; sorghum, 0/26; and cabbage, 0/8.

*Cephalosporium acremonium* Corda, received from J. F. Adams. Manns and Adams (31) and Reddy and Holbert (40) recently worked with this fungus. About 10 per cent of the wheat\* coleoptiles inoculated in tubes were infected; many callosities occurred. Results of greenhouse inoculations: corn\*, 5/13; barley, 0/10; and wheat, 0/14.

*Colletotrichum nigrum* E. & H., secured from C. L. Porter. It induced wheat\* coleoptiles to form callosities; many appressoria and some acervulae and spores were produced on some coleoptiles.

*Phytophthora cactorum* (Leb. & Cohn) Schr. from tulip stem rot; secured from O. A. Plunkett. It did not infect wheat in tubes. Results of greenhouse inoculations: cabbage\*, 6/13; radish, 0/16; barley, 0/30; broom corn, 0/15; sorghum, 0/11; wax bean\*, 13/14; and *Hemerocallis fulva*, 0/22.

*Botrytis cinerea* Pers.? from corn did not infect wheat in tubes. Results of greenhouse inoculations: sorghum, 0/29; wheat, 0/16; and radish, 0/7. *Cyclamen indicum* L. leaves were not infected, and apples were not rotted in the laboratory.

*Sclerotinia sclerotiorum* (Lib.) Mass. from parsnip did not infect wheat in tubes. Results of greenhouse inoculations: wheat, 2/7; sorghum\*, 5/17; cabbage, 0/20; and *Impatiens*\*, 6/15.

*Epicoccum tritici* P. Henn. from wheat glumes did not infect wheat or cabbage in tubes. Results of greenhouse inoculations: cabbage, 0/2; *Impatiens*\*, 4/4; wheat, 0/10; pop corn, 0/12; and sorghum, 4/9 (traces of leaf spots).

*Thielavia basicola* (B. & Br.) Zopf.; received from H. M. Fitzpatrick. None of the nearly 30 wheat coleoptiles inoculated in tubes were infected; sorghum and muskmelon seedlings were not infected. Results of greenhouse inoculations: broom corn, 0/16; barley, 0/13; and wax bean, 0/12. Apples were not rotted.

*Thielaviopsis paradoxa* (d. Seyn.) v. Höhn.; received from H. M.

Fitzpatrick. It did not infect wheat in tubes. In the greenhouse, 15 inoculations on broom corn and 11 on barley did not cause leaf spots.

*Pestalozzia funerea* Desm., secured from C. L. Porter, did not infect wheat coleoptiles, sorghum leaves, or apples.

*Chaetomium* sp. About 18 inoculations on wheat coleoptiles in tubes did not result in infections.

Two species of *Penicillium*, two species of *Aspergillus*, and *Sterigmatocystis niger* Van Tiegh. did not infect wheat in tubes. *Aspergillus* spp. did not rot apples. *Sterigmatocystis* did not infect tulip or *Impatiens*.

*Syncephalastrum nigricans* Vuill. did not infect wheat in tubes.

*Saprolegnia* sp. from a goldfish did not infect wheat in tubes; in the greenhouse, 18 inoculations on corn and 12 on barley failed to cause leaf spots.

*Cunninghamella elegans* Lendner? did not infect wheat in tubes and did not rot apples.

Four isolations of *Cephalothecium roseum* Cda. from peach fruit, walnut hulls, and cotton were used. Three of them did not rot wounded apples in dishes; one did not infect wheat or sorghum seedlings in tubes, and another did not infect wheat in tubes or dandelion leaves in the greenhouse.

*Fusarium* sp. from watermelon fruit did not cause corn leaf spots in 12 inoculations. *Fusarium* sp. from peach rot did not infect wheat in tubes or rot wounded apples.

#### DISCUSSION

Some of the results from the inoculations may be summarized on the basis of hosts as follows: Red Wave wheat was infected by *Diplodia zaeae*, *Cephalosporium acremonium*, *Colletotrichum nigrum*, and all cultures of *Alternaria*, *Macrosporium*, *Acrothecium*, and *Helminthosporium* placed on it. Many of the dematiaceous fungi infected barley, oats, rye, pop corn, and Yellow Dent corn; their callosities and leaf spots resembled those of wheat. Broom corn and sorghum were infected by many of the dematiaceous fungi; brown callosities and red or brown leaf and stem spots were the chief symptoms.

Many of the dematiaceous fungi infected cabbage, radish, and turnip. Prominent leaf spots and yellow callosities with circular margins appeared. The reactions of tomato seedling stems to infection by species of *Alternaria* and *Macrosporium* resembled those of cabbage; these fungi induced soybean seedling stems to form yellow callosities. Several species of dematiaceous fungi induced pumpkin and muskmelon seedling stems to form numerous large, complex callosities.

*Macrosporium iridis* and *M. parasiticum* directly penetrated the cells of onion bulb membranes; the other dematiaceous fungi used failed to do so. Callosities were not produced. Many dematiaceous fungi caused leaf spots in *Impatiens pallida*; aerial mycelium usually projected from the lower sides of the spots.

Lack of time made it impossible to apply Koch's rules to prove the pathogenicities of the fungi used in the inoculations. However, the 198 "new diseases" probably were new diseases caused by the particular fungi used in the inoculations, because: (a) aseptic seedlings in tubes were inoculated with pure cultures of fungi and exhibited symptoms of disease within 2-7 days after inoculation; (b) leaves of plants inoculated with pure cultures of fungi in the greenhouse exhibited prominent leaf spots 0.5-1 cm. in diameter within 2-7 days after inoculation; (c) these lesions appeared directly below the fungi used in inoculation and not elsewhere; and (d) while 198 "new diseases" appeared, 201 similar combinations of other flowering plants and fungi did not result in the appearance of diseases.

Plants that did not show infections usually were not reinoculated a sufficient number of times to prove that they were immune. However, the inoculations seem to justify the conclusion that Red Wave wheat coleoptiles in tubes were immune to *Thielavia basicola*, *Thielaviopsis paradoxa*, *Sterigmatocystis niger* from onion, *Phytophthora cactorum*, *Botrytis cinerea*?, and *Epicoccum tritici*. Barley leaves appeared to be immune to *Alternaria brassicae* f. *microspora* P.F. 1. Cowpea leaves seemed to be immune to *Helminthosporium gramineum*.

The cross inoculations showed that many species of *Alternaria* and *Macrosporium* and some of *Helminthosporium* are facultative parasites having wide host ranges. Although they infected a large number of plants and there was no apparent correlation between the sources of the fungi and the plants which they infected, their failures to infect many other plants showed that, under the conditions used, their experimental host ranges were limited. All species of *Alternaria* and *Macrosporium* placed on wheat coleoptiles in tubes infected them. *Helminthosporium gramineum* infected only 14 of the 46 kinds of plants inoculated with it. *Acrothecium* resembled *Alternaria* in its parasitism.

*Phytophthora cactorum*, *Sclerotinia sclerotiorum*, *Thielavia basicola*, and *Thielaviopsis paradoxa*, which are destructive parasites, failed to infect most of the plants on which they were placed. The *Penicillium* group of fungi, *Botrytis*, *Cephalothecium*, and *Fusarium* did not infect any of the plants inoculated with them. *Diplodia zeae* caused a serious disease of wheat coleoptiles (Pl. XXXVII, figs. 7-11).

The 198 "new diseases" which resulted from the cross inoculations exhibited symptoms ranging from isolated callosities and scattered brown host cells to prominent leaf spots and seedling stem spots and rots. These diseases occurred under conditions which are not apt to appear in fields. Aseptic seedlings in tubes and plants under covered bell jars are physiologically different from normal plants of the same varieties growing under field conditions.

The random cross inoculations have a direct bearing on the problem of susceptibility of plants to fungi. What is the real condition of the plants

at the time of infection and while the diseased lesions are enlarging? The cabbage inoculations furnish some information in answer to these questions. If, after cabbage plants had been covered with bell jars for three days, the bell jars were removed and the plants exposed, the leaves often began to wilt within 30 minutes and dried up within a few hours. In contrast to this, if the interiors of the bell jars were slowly dried out during a period of 1-2 days, the leaves usually retained their normal appearance and the cabbage plants grew well for weeks after inoculation. Obviously, the ability of the plants to protect themselves against loss of water was much different after they had been covered with bell jars for three days from what it was before they were covered and after the interiors of the bell jars had been dried slowly. The humidity of the air in the bell jars was so high that numerous droplets of water from guttation and condensation usually appeared and remained on the leaves. Cabbage, radish, and some other leaves were unusually easily bruised after they had been covered by bell jars.

Infections probably occurred within 24-36 hours after inoculation, which was before much change had occurred in cabbage leaves. However, all the leaf spots which occurred in the cross inoculations of all hosts developed very rapidly and often reached diameters of 0.5-1 cm. while the plants were covered and enlarged very little after the bell jars were removed. From a physiological standpoint, *perhaps the cabbage was really a different plant* during the second and third days under the bell jars. Possibly there is some correlation between susceptibility and such altered transpiration relations, for many plants were infected by *Alternaria* and *Helminthosporium* when they were covered by bell jars and only a few were infected by these fungi when they were placed in large glass cases and water was sprayed on to them. *This indicates that the bell jars did more to aid the infecting fungi than merely to prevent the evaporation of water placed on the leaves.*

Bell jars were covered with burlap sacks because the sun shining on them often raised the interior temperatures to 30-35° C., and the enclosed plants, when unprotected from the sunlight, frequently took on a scalded appearance. Even when so protected, *Commelina*, *Datura*, potato, tomato, tobacco, radish, peach, pea, and sugar beet leaves were often badly injured; infections in scalded leaves were not counted. Unless plants were so injured, most of their leaves continued to live after the bell jars were removed. Frequently some of the lower leaves of covered plants turned yellow; only a few spots occurring in yellow leaves were counted. In some radish leaves, only the large veins turned yellow. Leaves of covered radish plants that were dried too quickly often exhibited dry regions of tissues between the large veins.

The above descriptions of physiological reactions and accompanying conclusions are based chiefly on observations on cabbage and radish leaves;

however, they apply to a lesser extent to oats and to the other plants covered by bell jars. Additional information was secured when cowpea plants were covered for 3 days before and for 3 days after inoculation in an attempt to make them susceptible to *Helminthosporium gramineum*; no infections occurred.

Perhaps the occurrence of green leaf spots in leaves that have turned yellow is more common than is usually supposed. In the greenhouse inoculations, green leaf spots occurred in leaves that had turned yellow under the bell jars in the following cases: *Helminthosporium gramineum* infections of wheat and rye (Pl. XXXVII, figs. 2-4), *Alternaria brassicae* f. *microspora* P.F. 1 and *Alternaria tenuis* P.F. 1 infections of radish (Pl. XXXVII, fig. 5), and *Alternaria rugosa* P.F. 1 infection of *Solanum nigrum*; the leaves were green and appeared to be normal at the time of inoculation. Similar green infection spots were collected in Illinois in yellow apple leaves infected with *Gymnosporangium juniperi-virginianae* Schw., and green fruit spots were caused by this fungus in ripe yellow crab apples. Very similar is the phenomenon of green borders surrounding infection spots in yellow leaves. The following leaf spots of this type were collected in Illinois in 1924 and 1925: green borders surrounding the uredinial pustules of *Puccinia coronata* Cda. in yellow oat leaves, of *Puccinia triticea* Erik. in yellow wheat leaves, and of *Uromyces trifolii* (Hedw.) Lév. in yellow leaves of *Trifolium repens* L.; also green borders surrounding brown leaf spots caused by undetermined fungi (not rusts) in yellow leaves of apple, *Polygonum* sp., and *Prunus serotina* Ehrh. Green borders surrounded many leaf spots caused by *Cylindrosporium* in yellow leaves of *Prunus melanocarpa* (A. Nels.) Rydb. which were collected in Montana in 1925. The fungi considered here usually cause yellow or brown leaf spots.

Ward (58) described green margins surrounding uredinial pustules of *Puccinia dispersa* in bromes. Mains (30) reported similar spots caused by *Puccinia sorghi* in corn leaves. He says that, in section, the cells of the green regions appear to be normal except for the presence of haustoria in them, while in the neighboring yellow tissues the cells are shriveled and dead. Hayes, Stakman, and Aamodt (22 a) mention green islands in wheat leaves. Tischler (54) says that *Euphorbia* cells infected with *Uromyces* have higher osmotic pressures than healthy ones; the removal of sugar from the cells is affected. Tubeuf and Smith (56) give examples of diseased plant tissues which apparently draw food and water from undiseased tissues.

Perhaps the following theoretical explanation accounts for the formation of green leaf spots: The internal fungi seem to react to the yellowing of the leaves and, possibly by means of chemicals, stimulate chlorophyll-formation or delay its decomposition in the host tissues near the leaf spots. It is interesting to see facultative parasites like *Alternaria* and *Helminthosporium* act like the highly specialized rusts in this respect.

## SUMMARY

1. In the laboratory and greenhouse, 69 isolations of fungi were used in 5,369 inoculations of 78 species and varieties of flowering plants. Of these inoculations, 3,602 were made in the greenhouse, 1,633 were made in tubes, and 134 were made in dishes. Thirty-nine cultures of *Alternaria* and *Macrosporium* were used in 2,258 of the greenhouse inoculations and 1,165 of the tube inoculations.

2. Of the 198 "new diseases," 165 resulted from inoculations with cultures of *Alternaria* and *Macrosporium*; wheat seedlings were infected by all species of *Alternaria* and *Macrosporium* placed on them.

3. In using the same kinds of unit fungus-flowering plant combinations of inoculations, cultures of *Alternaria* and *Macrosporium* failed to cause diseases in 101 cases and other fungi failed in 100 cases.

4. Cross inoculations showed that *Helminthosporium gramineum* and many species of *Alternaria* and *Macrosporium* have wide experimental host ranges.

5. *Alternaria*, *Macrosporium*, and *Helminthosporium* usually infected the plants here considered within 1-7 days after inoculation or not at all.

6. *Helminthosporium* sp. from barley leaves was one of the most virulent parasites used. In tubes, wheat coleoptiles exhibited new sporophores and spores within 4 days after inoculation; spores appeared on barley leaf spots in the greenhouse within 3 days after inoculation.

7. *Impatiens pallida* leaves were infected by many species of *Alternaria*; mycelium often projected into the air on the lower sides of the leaf spots.

8. Most of the diseases were not serious; they are of value as additions to a knowledge of the experimental host ranges of the fungi used.

9. *Aspergillus*, *Botrytis*, *Cephalothecium*, *Chaetomium*, *Cunninghamella*, *Epicoccum*, *Fusarium*, *Penicillium*, *Pestalozzia*, *Phytophthora*, *Saprolegnia*, *Sclerotinia*, *Sterigmatocystis*, *Syncephalastrum*, *Thielavia*, and *Thielaviopsis* did not penetrate unbroken wheat coleoptiles.

10. *Acer*, *Allium*, *Callistephus*, *Citrus*, *Commelina*, *Cyperus*, *Hemerocallis*, *Ipomoea*, *Lamium*, *Linum*, *Nicotiana*, *Ornithogalum*, *Podophyllum*, *Smilacina*, *Vinca*, and *Viola* leaves were not infected by any of the fungi placed on them.

11. None of the fungi placed in wounds in apples caused distinct rots.

12. Green leaf spots occurred in leaves that had turned yellow under the bell jars in the infections of wheat and rye by *Helminthosporium gramineum*, infections of radish by *Alternaria brassicae* f. *microspora* P.F. 1 and *A. tenuis* P.F. 1, and infection of *Solanum nigrum* by *A. rugosa* P.F. 1.

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## EXPLANATION OF ILLUSTRATIONS

## PLATE XXXVI

Solid lines connecting circles mean that infections occurred; broken lines mean that infections did not occur. In the small circles, "T" refers to inoculations of seedlings in tubes, "G" refers to leaf inoculations in the greenhouse, and "L" refers to inoculations in dishes. In the fractions, the denominator represents the number of inoculations and the numerator represents the number of infections (leaf spots or infected seedlings). Most of the greenhouse fractions are exact counts; the tube fractions are close estimates.

## PLATE XXXVII

FIG. 1. Photomicrograph of the surface of a wheat coleoptile 7 days after inoculation with *Alternaria ribis*. E, plain elongate callosity. T, toothed elongate callosities. C, circular-margined callosity. R, radiate callosity. Each callosity contains a penetration hypha. The upper brown wheat cell is 25 microns wide.

FIGS. 2-4. Photographs of wheat leaf spots caused by *Helminthosporium gramineum* taken 3 days after inoculation. Figures 2 and 3 show green spots in yellow leaves; figure 4 shows a yellow spot in a green leaf.

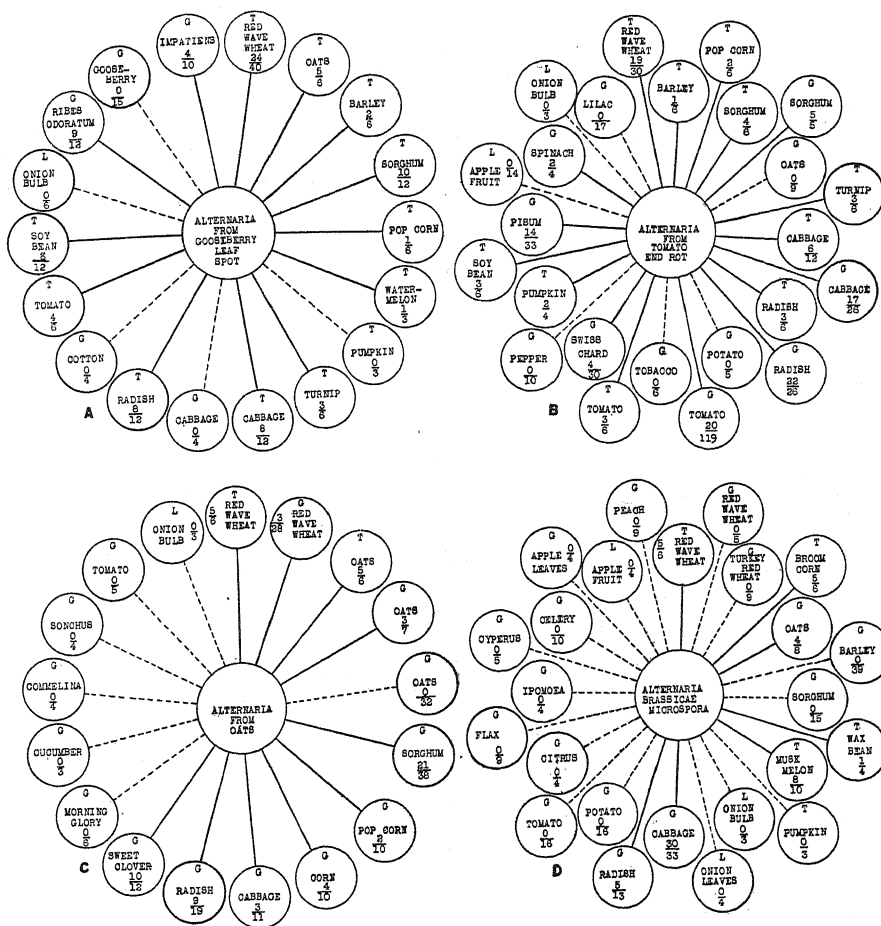
FIG. 5. Photograph of a green leaf spot caused within 4 days after inoculation by *Alternaria brassicae* f. *microspora* P.F. 1 in a radish leaf which turned yellow under the bell jar.

FIG. 6. Photograph of a sorghum leaf taken 3 days after inoculation with *Alternaria* (*Macrosporium avenae*?) showing the auto-stained red infection ring under the ring of spores.  $\times 2$ .

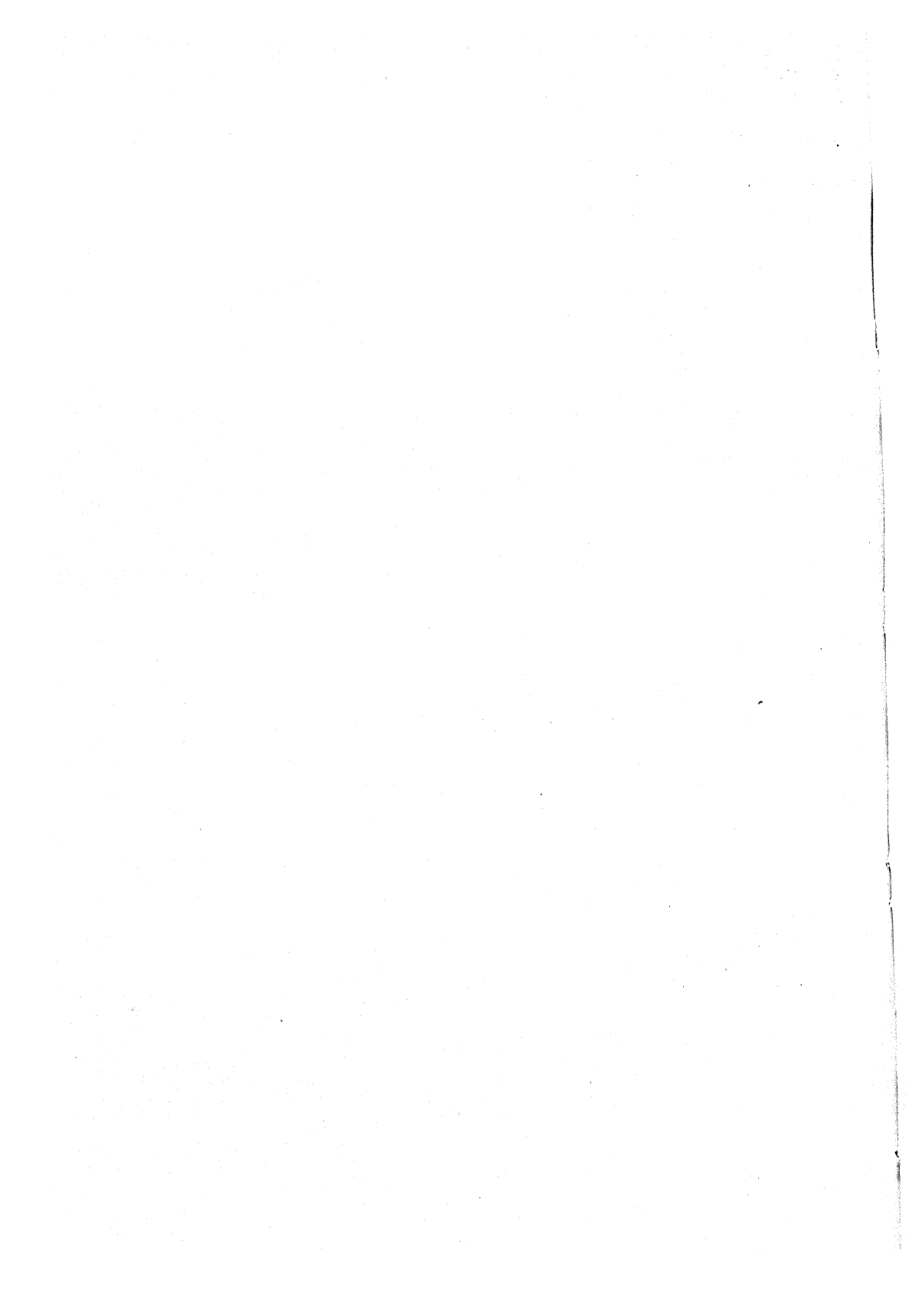
FIG. 7. Drawing of surface view of a wheat coleoptile 7 days after inoculation with *Diplodia zeae* showing callosities, C, and free penetration hyphae, P. Stippling represents host protoplasm. Lowest cell is about 20 microns wide.

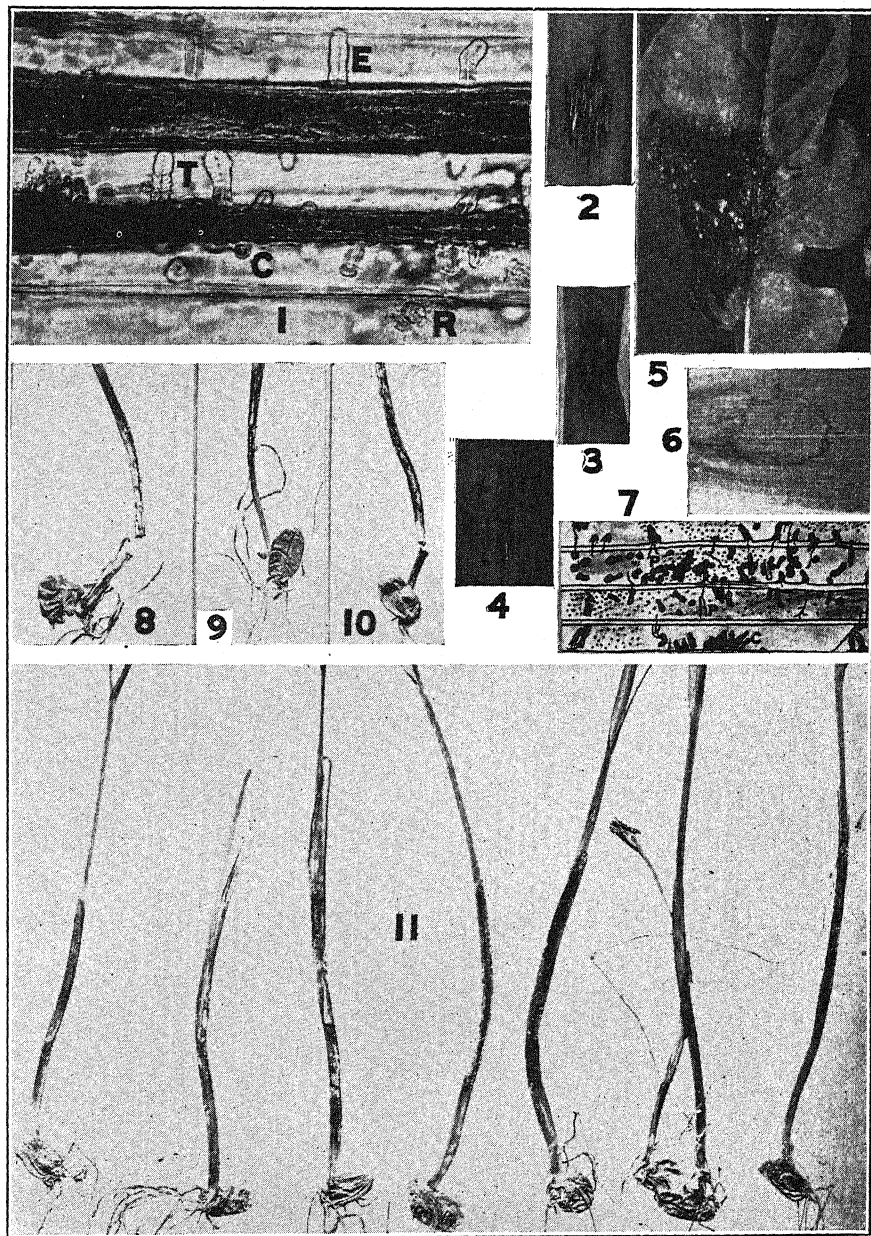
FIGS. 8-10. Photographs of wheat seedlings from a tube culture 7 days after inoculation with *Diplodia zeae*, showing white mycelium on the deeply browned, rotted coleoptiles.

FIG. 11. Like figure 8, except that seedlings were photographed 11 days after inoculation.



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## REVIEWS OF SOME PERENNIAL LUPINES I. CALCARATI-LAXIFLORI

CHARLES PIPER SMITH

(Received for publication October 28, 1925)

Agardh (1835) divided the genus *Lupinus* into 12 groups, using the name *Sericei* for his "Tribe I," with 4 subdivisions indicated as follows:

*Stipulis subnullis, racemo laxifloro, floribus purpureis, labio superiore calycis plerumque saccato.*

43. *L. perennis* L., 44. *L. tenellus* Dougl., 45. *L. laxiflorus* Dougl.

*Stipulis subulatis, racemo laxifloro, floribus coeruleis vel albidis, labio superiore calycis estrumoso.*

46. *L. argyraeus* Dec., 47. *L. argenteus* nob., 48. *L. ornatus* Dougl., 49. *L. leucopsis* nob.

*Floribus in racemo crasso densis flavis.*

50. *L. albicaulis* Dougl., 51. *L. Sabinianus* Dougl., 52. *L. sulphureus* Dougl., 53. *L. sericeus* Pursh.

*Floribus in spica densissima albidis, roseo tinctis.*

54. *L. aridus* Dougl., 55. *L. leucophyllus* Lindl., 56. *L. plumosus* Dougl., 57. *L. Chamissonis* Esch.

Translated freely and rearranged in the form of a present-day key, the above may be more easily surveyed:

Flowers loosely arranged.

Flowers purple, upper calyx-lip usually saccate; stipules greatly reduced.....

*perennis, tenellus, laxiflorus.*

Flowers blue or whitish, upper calyx-lip not gibbose; stipules subulate

*argyraeus, argenteus* Agardh, *ornatus, leucopsis.*

Flowers crowded together.

Flowers yellow, densely racemose...

*albicaulis, Sabinii, sulphureus, sericeus.*

Flowers white, rose-tinted, densely

spicate..... *aridus, leucophyllus, plumosus, Chamissonis.*

All the species named above are North American excepting *L. argyraeus*, for which Agardh was unable to give the original locality.

Based primarily upon the development of ligneous tissue in the stems, Agardh distinguished as his ninth division, Tribe K, the *Paniculati*. Six of the 11 species there included are North American, namely: *L. albifrons*

[The *Journal* for October (13: 465-520) was issued October 15, 1926.]



Benth., *L. Douglasii* Agardh, *L. flexuosus* Lindl., *L. macrocarpus* H. & A., *L. littoralis* Dougl., and *L. decumbens* Torr.

Piper (1906) was the first to follow Agardh in arranging the lupines into groups, and in his group Sericei he accounted for 14 species and 4 varieties as known to him from the state of Washington. These species are: *lyallii*, *aridus*, *minimus*, *lepidus*, *piperi*, *subsericeus*, *littoralis*, *albicaulis*, *leucophyllus* (with *plumosus* as a variety), *canescens*, *suksdorfii*, *sericeus*, *ornatus*, and *alpicola*. Six of these species were unknown to Agardh, while 2, *minimus* and *lepidus*, were included by Agardh in his Tribe F, Polyphylli, and one, *littoralis*, was assigned to his Tribe K, Paniculati. For *L. laxiflorus*, Piper proposed a group Calcarati, and for *sabinii* and *sulphureus* a group Sulphurei, with which, however, he did not associate *albicaulis* and *sericeus*, as did Agardh.

Heller (1910), in treating the lupines of Nevada, recognized three groups in placing the species that would naturally fall into Agardh's tribe Sericei. His Cespitosi included *alpinus* sp. nov., *pinetorum* sp. nov., *sellulus*, and *confertus*; his Calcarati, *calcaratus* and *caudatus*; his Ecalcarati, *andersoni*, *nevadensis* sp. nov., *montigenus* sp. nov., and *meionanthus*.

To locate the lupines of this relationship in Rydberg's latest treatment (1917), one must examine his groups Sulphurei, Calcarati, Argophylli, Albicaules, Foliosi, Ornati, Lepidi, Cyanei, Leucophylli, Candicantes, Monticolae, and Caespitosi. He discarded the name Sericei, placing *L. sericeus* in his group Cyanei.

My studies permit me to agree with others that Agardh's tribe Sericei needs to be subdivided and the species to be rearranged. I do not see any close relationship between *L. perennis* and *tenellus*, although the latter is allied to *laxiflorus*. Also, *albicaulis* and *sulphureus* are distinctly different from each other, and neither should be in the same group with *sabinii* and *sericeus*. Likewise, *aridus* and *chamissonis* are radically different from each other, neither having any apparent affinity with *leucophyllus* and *plumosus*. Readjusting, therefore, in line with my own conclusions, Agardh's Tribe I gives place to 6 primary groups, the Sericei of my arrangement including several species not known to Agardh and 4 of the North American species of his Tribe K, Paniculati. These 6 groups are now differentiated by the following key:

Apex of banner normally not much reflexed from upper margin of wings, the ventral median sulcus of banner deep, including much of the wings.

Pedicels short and stout, usually less than 3 mm. long; floral bracts usually persistent or at least tardily deciduous; racemes subspicate, flowers usually crowded; banner pubescent on the back.....LEUCOPHYLLI.

Pedicels usually 3-12 mm. long; floral bracts usually early deciduous; flowers distinctly racemose; banner pubescent or glabrous on the back.....CALCARATI.

Apex of banner normally well reflexed from upper margin of wings; ventral median sulcus usually shallow, including very little of the wings.

Pedicels short and stout, usually less than 3 mm. long; floral bracts usually persistent or tardily deciduous; racemes subspicate and flowers usually crowded; banner glabrous. . . . . LEPIDI.

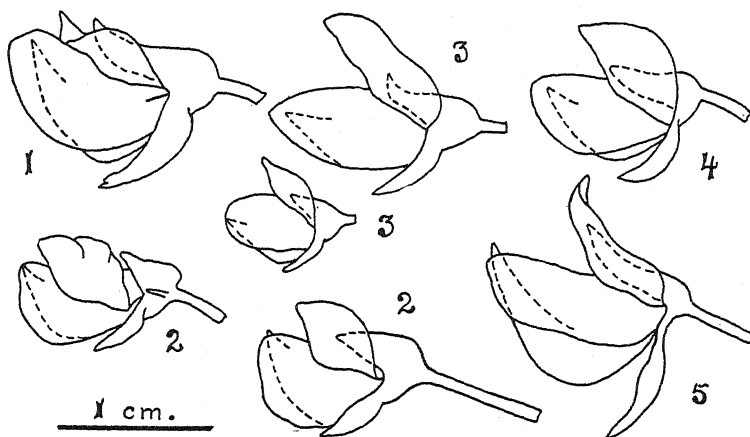
Pedicels usually 3-12 mm. long; floral bracts early deciduous; flowers seldom crowded.

Leaflets glabrous or glabrate above; keel ciliate; banner glabrous. . . PERENNES.

Leaflets permanently pubescent above.

Keel more or less ciliate on upper edges; banner pubescent on the back, or lower petioles elongated. . . . . SERICEI.

Keel non-ciliate and petioles all short; banner rarely pubescent on the back. . . . . ALBICAULES.



TEXT FIG. 1. 1. *Leucophylli*. 2. *Calcarati*. 3. *Lepidi*. 4. *Sericei*. 5. *Albicaules*.

Text figure 1, showing side views of representative flowers, is here introduced to assist one in using the above key. I expect to discuss these groups as time and conditions permit, but now we shall consider only the *Calcarati*, my conception of which is quite different from that of Piper (1906), Heller (1910), or Rydberg (1917). Indeed, the group is a large and complex one, and I find it desirable to provide a secondary division of it, both for the sake of ordinary convenience and to express my ideas as to the interrelationships of the species concerned. As I now understand these forms, 3 subdivisions accommodate the need suggested, and the following key characterizes and names the minor groups indicated.

Wing-petals pubescent on outer surface near upper distal angle, or banner long-clawed, upper calyx-lip largely exposed, and calyx-cup spurred. . . . . CALCARATI-LAXIFLORI.

Wing-petals without pubescence near upper distal angle, although often with a few villi near claws or along one of the median veins.

Leaflets permanently hairy above. . . . . CALCARATI-SULPHUREI.

Leaflets glabrous or glabrate above. . . . . CALCARATI-PARVIFLORI.

As I see it, the species that are nearest *L. sulphureus* do not have yellow petals, and the relative development of the calyx-spur has been over-emphasized. Discussion of all three of these subdivisions can not be accomplished in the present paper, the following pages treating only the first mentioned.

#### CALCARATI-LAXIFLORI

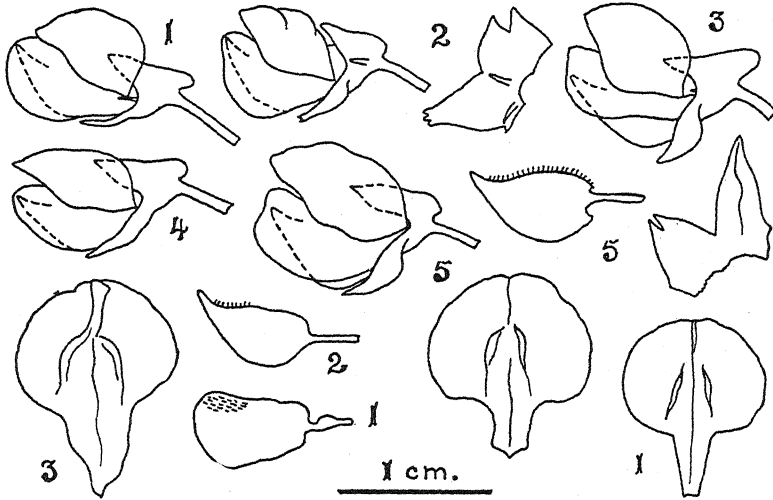
Five proposed species belong here, and these I have considered and compared many times. They are certainly very closely related and easily vary into one another to such an extent that I often question my own action in here acknowledging two species.

#### Key to Species and Varieties

- Leaflets glabrous or glabrate above.....2. *L. pseudoparviflorus*.
- Leaflets permanently pubescent above.
  - Banner wider and shorter-clawed, its reflexed sides covering most of the upper calyx-lip; keel ciliate; calyx spurred.
    - Pubescence loose, widely spreading on pedicels and lower portion of stems; floral bracts subpersistent....1 f. var. *villosulus*.
    - Pubescence appressed generally; floral bracts early deciduous.....1 d. var. *cognatus*.
  - Banner narrower and longer-clawed, its reflexed sides covering little or none of the upper calyx-lip.
    - Floral bracts subpersistent; calyx merely gibbous; keel non-ciliate.....1 e. var. *durabilis*.
    - Floral bracts caducous; calyx spurred; keel usually ciliate.
      - Spur over 1 mm. long.....1 c. var. *calcaratus*.
      - Spur not over 1 mm. long.
        - Flowers 8-9 mm. long.....1 b. var. *silvicola*.
        - Flowers 10-12 mm. long.....1 a. *L. laxiflorus*.

Heller (1911) has reported his difficulties in establishing the true status of *L. laxiflorus*. Both the original description and plate call for an obcordate banner, a character certainly not conspicuous in any of the common Columbia River lupines of today. Also the plate does not nicely represent the few Douglasian specimens now in this country, and reasonable doubt exists as to the identity of the specimens (evidently garden productions) used by the artist. The description also calls for a non-ciliate keel and a saccate upper calyx-lobe, a combination not commonly found in the region concerned, yet occasionally met with, as at Salem, Oregon [M. E. Peck 2312 and 2314 (WSO)]. For some time, therefore, I have wondered as to just what type of plant actually prompted Douglas' field description. It seems reasonable to assume that he must have had before him at the time exceptional plants not at all typical of the species. Thus it becomes practically impossible to identify this species with satisfaction from the plate and description; but fortunately there persists a specimen, collected

by Douglas and labeled by himself or Lindley, deposited in the Lindley Herbarium at Cambridge University, England. Thanks to Miss Alice Eastwood, we have a fine photograph of this specimen, also copy of Miss Eastwood's notes, taken with the specimen before her. This photograph distinctly portrays a species now common in the region of the Columbia River drainage, and the specimen can very logically be accepted as the



TEXT FIG. 2. 1. *Lupinus laxiflorus* Douglas. T. Howell 1494 (US). 2. *L. laxiflorus* Douglas. S. P. Sharples 136 (US). 3. *L. laxiflorus calcaratus* (Kellogg) C. P. Smith. A. A. Heller 9744 (CPS). 4. *L. laxiflorus silvicola* (Heller) C. P. Smith. A. A. Heller 9857 (CPS). 5. *L. laxiflorus cognatus* C. P. Smith. P. A. Munz 4607 (CPS).

"type" of *L. laxiflorus* Dougl. Further, Miss Eastwood's photograph of the Lindley Herbarium specimen labeled "*L. arbustus* Dougl." (likewise logically the type of that species) shows that to be the same species as the specimen marked *L. laxiflorus*. In both of these the keel is ciliate, as accredited to *L. arbustus* by Douglas' notes, according to Lindley (1829). Fortunately this is the species most commonly identified as *L. laxiflorus* by the botanists of the region concerned. As in so many other lupine species, the leaflets in this vary greatly in length and width, and in the density of their pubescence.

1 a. *LUPINUS LAXIFLORUS* Dougl. Lindl. Bot. Reg. 14. Pl. 1140. 1828.

*Lupinus arbustus* Dougl. Lindl. Bot. Reg. 15. Pl. 1230. 1829.

*Lupinus laxiflorus montanus* Howell. Erythea 3: 33. 1895.

*Lupinus laxiflorus theiochrous* Robinson. Piper, Contr. U. S. Nat. Herb. 11: 358. 1906.

Plants 3-7 dm. tall, stems fibrous, usually several from the woody perennial rootstock, mainly appressed-pubescent; leaves few, long-petioled,

hairy or glabrate above, petioles slender, 6-22 cm. long, stipules small, narrow, leaflets 7 to 13, sublinear to oblanceolate or broadest near the middle, acute, 30-90 mm. long by 3-20 mm. wide; peduncles 3-6 cm. long, racemes 8-15 cm. long, lax or otherwise; flowers subverticillate or scattered, 10-12 mm. long, bracts 5-7 mm. long, sublinear, usually early deciduous, pedicels slender, usually 3-10 mm. long, subappressed- or spreading-pubescent; calyx often bracteolate, loosely pubescent, upper lip notched, short and broad, much shorter than the lower, which is entire or 3-toothed, calyx-cup usually spurred (produced backward above the pedicel); petals blue, white, pink, yellow, or lilac, often the wings pink and the banner blue with yellow center changing to violet, banner with prominent claw, more or less pubescent on the back, wings usually pubescent on outer surface near upper distal angle, keel ciliate on upper edges or rarely non-ciliate, only the acumen curved; pods subappressed-pubescent, 25-40 mm. long, 7-10 mm. wide, ovules three to six, seeds dark reddish or brown, unmarked or obscurely mottled, 4-5 mm. long and almost as broad.

Apparently the most constant and noteworthy diagnostic character of this species is the rather inconspicuous pubescence near the upper distal angle of the wing-petals, a character seemingly unique among North American lupines. Occasionally this pubescence is absent when every other character requires the placing of the specimen in *L. laxiflorus*. Some specimens which agree with the species *sensu strictiore* are:

WASHINGTON. ASOTIN COUNTY: Grande Ronde River canyon, opposite Zindel, May 20, 1922, H. St. John and R. H. Brown 3225 (SCW). CHELAN COUNTY: Wenatchee Mountains, June 2, 1896, K. Whited 120 (UO); same, July 4, 1903, J. S. Cotton 1301 (SCW). KITTITAS COUNTY: Head of Perkins Creek, June 21, 1904, J. S. Cotton 1608 (SCW); near Mt. Baldy, Sept. 11, 1904, J. S. Cotton 1750 (SCW); Swauk River valley, 1913, S. P. Sharples 136 (CA, US). KLINKITAT COUNTY: Klickitat River, July 25, 1899, J. B. Flett 1256 (SCW); "western" part, June 2 and July 3, 1894, W. N. Suksdorf 2306 (CPS, UC, UO); near Columbus, April 23, 1909, W. N. Suksdorf (CPS); Goldendale, April 26, 1920, G. L. Zundel (CPS); Maryhill, April 28, 1920, G. L. Zundel (CPS); Trout Lake, June, 1923, G. A. Pearson (CPS). YAKIMA COUNTY: Ahtanum Creek, July 15, 1923, E. Nelson (CPS).

IDAHO. CUSTER COUNTY: Mackay, Aug. 1, 1911, A. Nelson and J. F. McBride 1530 (DS, PC). GEM COUNTY: Sweet, Squaw Creek, May 9, 1911, J. F. McBride 844 (DS, PC). IDAHO COUNTY: Craig Mountains, May 22, 1892, Sandberg, McDougal, and Heller 226 (DS, PC); Camas Prairie, near Grangerville, June 30, 1901, L. F. Henderson 2732 (DS). LATAH COUNTY: Genesee, 1923, F. A. Warren (CPS). NEZ PERCE COUNTY: Lewiston, June 10, 1896, A. A. and E. G. Heller 3224 (DS, SCW); Mission Creek, May 30, 1924, H. St. John, F. A. Warren, and J. A. Cary 3247 (CPS).

OREGON. BAKER COUNTY: Powder River Mountains, Aug., 1896, W. C. Cusick (UO); same, Aug., 1896, C. V. Piper (SCW). BENTON COUNTY: Corvallis, June 19, 1898, T. Kincaid (SCW). CLACKAMAS COUNTY:

Oswego Lake, May 24, 1919, M. E. Peck (WSO). CROOK COUNTY: Ochoco Creek, Prineville-Mitchell road, June 30, 1919, R. S. Ferris and R. Duthie (DS). GRANT COUNTY: Strawberry Mountains, July 3, 1919, R. S. Ferris and R. Duthie 819 (DS). HARNEY COUNTY: Riley, May, 1914, Mrs. R. D. Cooper (CPS, WSO). HOOD RIVER COUNTY: Mt. Hood, Aug. 10, 1893, T. Howell 1494<sup>1</sup> (DS, UC, UO); Hood River, May 26, 1910, A. A. Heller 10099 (CPS); same, June 23, 1911, M. E. Peck 2311 (WSO). LANE COUNTY: Summit, Horse Pasture Mountain, July 1, 1914, M. E. Peck 5481 (WSO); Eugene, May 21, 1920, R. V. Bradshaw 1597 (DS). MARION COUNTY: Salem, May 28, 1910, M. E. Peck 2312 and 2314 (WSO); same, May 13, 1916, J. C. Nelson 567 (DS); same, May 19, 1921, J. C. Nelson 3655 (CPS). MULTNOMAH COUNTY: Portland, July 10, 1902, E. P. Sheldon 10858 (PC, SCW); Booneville, June 14, 1903, E. P. Sheldon 12103 (DS, UO). SHERMAN COUNTY: Kent, July 1, 1921, M. E. Peck 9972 (WSO). UNION COUNTY: Hills of lower Powder River, May 22, 1901, W. C. Cusick 2514 (PC, SCW, UO). WALLOWA COUNTY: Trout Creek, Sept., 1902, W. C. Cusick 2131 (UO); Wallowa Mountains, July 29, 1907, W. C. Cusick 3187 (SCW, UO); same, 1911, W. C. Cusick 3690 and 3691 (CA); Imnaha, May 30, 1923, W. Sherwood (CPS). WASCO COUNTY: The Dalles, May, 1888, T. Howell (UO); same, May 19, 1913, W. N. Suksdorf (CPS).

CALIFORNIA. MODOC COUNTY: Rocky slopes, June 18, 1893, M. S. Baker (UC).

1 *b.* LUPINUS LAXIFLORUS SILVICOLA (Heller) C. P. Smith. Jepson, Man. Pl. Cal. 527. 1925.

*Lupinus silvicola* Heller. Muhlenbergia 6: 81. 1910.

Flowers only 8-9 mm. long, calyx-spur scarcely 1 mm. long, petals mostly blue, tinged with violet.

WASHINGTON. KLICKITAT COUNTY: Trout Lake, June, 1923, G. A. Pearson (CPS, SCW).

OREGON. GILLIAM COUNTY: Hoover Creek, June 1, 1894, J. B. Leiberger 143 (UC, US). KLAMATH COUNTY: Williamson River, July, 1913, Mrs. A. L. Coombs (CA). LANE COUNTY: Horse Pasture Mountain, July 1, 1914, M. E. Peck 5481 (CPS).

NEVADA. STOREY COUNTY: Virginia City, June, 1885, T. S. Brandegee (CA).

CALIFORNIA. ALPINE COUNTY: Carson Spur, July, 1892, G. Hansen 364 (DS, UC). BUTTE COUNTY: Chico Meadows, June 25, 1915, A. A. Heller 12016 (CA, DS, UCX); summit above Jonesville, July 29, 1917, A. A. Heller 12853 (CA, DS, UCX). ELDORADO COUNTY: Echo Lake, Aug. 9, 1916, A. A. Heller 12515 (CA, DS, UCX); Emerald Bay, June-July, 1912, Helen D. Geis 103 (UC); Glen Alpine Spring, July 19, 1909,

<sup>1</sup> Type collection of Howell's variety.

Laura M. Lathrop (DS); Mt. Tallac, July 29, 1911, L. R. Abrams 4845 (DS). LASSEN COUNTY: Susanville, July 6, 1892, T. S. Brandegee (CA); Lassen Butte, Aug. 22-26, 1912, A. Eastwood 1804 (CA, CPS). MARIPOSA COUNTY: Inspiration Point, Sept. 1, 1894, W. R. Dudley (DS); same, June 5, 1897, J. W. Congdon (DS). NEVADA COUNTY: Donner Lake, July 19, 1893, E. L. Greene (DS, WLJ); same, July 16, 1903, A. A. Heller 6944 (CPS, DS, PC, UC); Frog Lake, Mt. Stanford, July 31, 1897, C. F. Sonne (UC); Independence Lake, July, 1903, Hall and Babcock 4582 (PC, UC); Truckee, July, 1895, C. F. Sonne (PC). PLACER COUNTY: Cisco, June 18, 1870, A. Kellogg (CA); same, H. M. Hall 8730 (UC); Deer Park, June 15-19, 1912, A. Eastwood 405 (CA, CPS); Squaw Valley, June 19, 1916, L. S. Smith 593 (UCX); Summit, July 16, 1909, A. A. Heller 9857 (CPS, DS, PC, SCW, UC, UCX). PLUMAS COUNTY: Big Meadows, 1886, Mrs. R. M. Austin (UC); Gold Lake region, Aug., 1917, Mrs. E. C. Sutcliffe (CA); Long Lake, July 10, 1912, H. M. Hall 9328 (UC); Mt. Hough, July 19, 1921, C. P. Smith 3449 (CPS). SIERRA COUNTY: Little Truckee River, July, 1903, E. B. Babcock 4527 (UC). SISKIYOU COUNTY: Gooseneast foothills, June 22, 1909, G. D. Butler 917 (DS); Klamath hills, May 14, 1909, G. D. Butler 705 (DS). TEHAMA COUNTY: Morgan, Mill Creek canyon, July 1, 1903, Hall and Babcock 4329 (UC).

*LUPINUS LAXIFLORUS CALCARATUS* (Kellogg) C. P. Smith. Bull. Torrey Bot. Club 51: 304. 1924.

*Lupinus calcaratus* Kellogg. Proc. Cal. Acad. 2: 195. 1862.

*Lupinus multinctus* A. Nels. Bot. Gaz. 53: 221. 1912.

*Lupinus variegatus* Heller. Muhlenbergia 8: 89. 1912.

Flowers 11-14 mm. long, calyx-spur 1-3 mm. long, petals blue, violet, rose, yellow, or white.

IDAHO. ADA COUNTY: Boise, May 26, 1911, June A. Clark 13 (DS). BLAINE COUNTY: Gelena summit, Aug. 12, 1916, McBride and Payson 3719 (CA); Ketchum, July 20, 1911, A. Nelson and McBride 1229 (DS). CANYON COUNTY: Big Willow, May 26, 1910, J. F. McBride 114 (SCW). NEZ PERCE COUNTY: Lake Waha, June 29, 1896, A. A. and E. G. Heller 3333 (DS). WASHINGTON COUNTY: Weiser, April 18, 1900, M. E. Jones 6249 (UO, US).

OREGON. GILLIAM COUNTY: Pine Creek, June 7, 1894, J. B. Leiberg 176 (UC, UO). HARNEY COUNTY: Steins Mountains, June 2, 1885, T. Howell (CA, UO). MALHEUR COUNTY: Brogan, 1910, Mrs. R. D. Cooper (CPS, WSO). UNION COUNTY: Eastern part, May 26, 1898, W. C. Cusick, 1896 (PC, SCW, UC, UO); hills of lower Powder River, May 22, 1901, W. C. Cusick 2514 (SCW, UC, UO, US). COUNTY NOT DETERMINED: Bendire Hill, June 15, 1896, J. B. Leiberg 2288 (PC, UC, UO); Santa Rosa Mountains, July 12, 1898, W. C. Cusick 2132 (UO).

CALIFORNIA. ELDORADO COUNTY: Glenbrook, June 28, 1900, W. R. Dudley (DS); Glenbrook summit, July 24, 1911, K. Brandegee (UC). INYO COUNTY: Long Valley, July 16, 1913, K. Brandegee (UC). MODOC COUNTY: Goose Lake Valley, May, 1894, Mrs. R. M. Austin (DS, US); Hills, June, 1898, Mmes. Austin and Bruce 2146 (CA, UC); Madeline Plains, June, 1892, Austin and Bruce 2144 (DS, UC); Parker Creek, June 14, 1919, R. S. Ferris and R. Duthie 76 (DS). TUOLUMNE COUNTY: Deadman Creek, July 26, 1915, W. L. Jepson 6572 (CPS).

NEVADA. DOUGLAS COUNTY: Spooner, June 23, 1902, C. F. Baker 1134 (CA). ELKO COUNTY: Deeth, July 8, 1912, A. A. Heller 10550 and 10551 (CA, DS, PC, UCX). ORMSBY COUNTY: Kings Canyon, June 1 and July 1, 1902, C. F. Baker 918 (DS, PC); same, C. F. Baker 923 (CA, PC, UC). STOREY COUNTY: Virginia City, K. Brandegee (DS). WASHOE COUNTY: Glencoe, June, 1891, M. E. Jones (UC); Franktown, June 26, 1882, M. E. Jones 3815 (CA); ridge south of Alum Creek, June 11, 1909, A. A. Heller 9744 (CPS, DS, SCW); same, Heller 9745 (CPS, DS); Lake Marlette, July, 1903, Hall and Chandler 4589 (UC); Mt. Rose, Aug. 17, 1905, P. P. Kennedy 1187 (DS, UC, UCX); Mt. Rose, Aug. 26, 1911, A. A. Heller 10342 (CA, CPS, DS, PC, UO).

UTAH. JUAB COUNTY: Mt. Ibapah, 1891, M. E. Jones (DS); same, June 17, 1903, M. E. Jones (UC, US).

1 d. *LUPINUS LAXIFLORUS COGNATUS* C. P. Smith. Jepson, Man. Pl. Cal. 527. 1925.

Plantae pubescentes pilis plurimum adpressis; bracteis floreis cito caducis; calyce paulo calcarati, labio superiore prope aperto lateribus reflexis vexilli; carina ciliata.

Mainly appressed-pubescent; floral bracts early deciduous; calyx-cup short-spurred, the upper lip largely covered by the reflexed sides of the broad banner; keel ciliate.

OREGON. BAKER COUNTY: Cornucopia, Aug. 30, 1915, M. E. Peck 5439 (WSO). GRANT COUNTY: Strawberry Mountains, July 15, 1921, M. E. Peck 10263 (CPS, WSO). KLAMATH COUNTY: Klamath Valley, 1864, H. M. Cronkhite 80 (UC). WALLOWA COUNTY: Wallowa Mountains, July 29, 1907, W. C. Cusick 3187 (TYPE, DS 117235; type-duplicates, UC, UO). COUNTY NOT DETERMINED: Highest Blue Mountains, Aug., 1897, W. C. Cusick 1716 (UC); Blue Mountains, June 24, 1910, W. C. Cusick 3469 (UO).

CALIFORNIA. MODOC COUNTY: Rocky slopes, June 18, 1893, M. S. Baker (DS). LOS ANGELES COUNTY: Swartout Valley, San Antonio Mountains, June 17, 1921, P. A. Munz 4607 (CPS).

1 e. *Lupinus laxiflorus durabilis* var. nov.

Plantae pubescentes pilis adpressis; bracteis floreis subpermanentibus; calyce gibbosi vix calcarati, labio superiore fere inoperto; carina eciliata.

Pubescence appressed; floral bracts subpersistent; calyx merely gibbose, the upper lip exposed; keel non-ciliate.



IDAHO. BONNE COUNTY: Priest River Range, July 17, 1897, J. B. Leiberger 2731 (UO).

1 f. *Lupinus laxiflorus villosulus* var. nov.

Plantae pubescentes laxe pilis pedicellorum cauliumque pendentibus; bracteis floreis subpermanentibus; calyce calcarati labio superiore prope aperto lateribus reflexis vexilli; carina ciliata.

Pubescence loose, widely spreading on pedicels and lower portions of stems; floral bracts subpersistent; calyx spurred, the upper lip mostly covered by the reflexed sides of the banner; keel ciliate.

NEVADA. ELKO COUNTY: Clover Mountains, near Deeth, July 22, 1908, A. A. Heller 9098 (DS 15620).

2. *LUPINUS PSEUDOPARVIFLORUS* Rydb. Mem. N. Y. Bot. Gard. 1: 232. 1900.

Seems to differ from *L. laxiflorus* in no fundamental points excepting that the upper surface of the leaves is glabrous or nearly so. Perhaps it should be reduced to varietal rank under that species.

MONTANA. FLATHEAD COUNTY: Big Fork, June 19, 1904, W. W. Jones (DS, UC). GALLATIN COUNTY: Bridger Mountains, June 17, 1897, Rydberg and Bessey 4441 (SCW); near Boseman, June-July, 1905, J. W. Blankinship 131 (DS).

IDAHO. NEZ PERCE COUNTY: Lake Waha, July 2, 1896, A. A. and E. G. Heller 3358 (UC).

ABBREVIATIONS

The following list will identify the abbreviations here used in citing the specimens recorded:

- CA, California Academy of Science, San Francisco.
- CPS, herbarium of the writer.
- DS, Dudley Herbarium, Stanford University.
- PC, Pomona College, Claremont, California.
- SCW, State College of Washington, Pullman.
- UC, Department of Botany, University of California.
- UCX, Division of Agronomy, University of California Experiment Station.
- UO, University of Oregon, Eugene.
- US, United States National Herbarium.
- WLJ, herbarium of Dr. W. L. Jepson, Berkeley, California.
- WSO, Willamette University, Salem, Oregon.

SAN JOSE, CALIFORNIA

LITERATURE CITED

- Agardh, J. G. 1835. Synopsis generis lupini.
- Heller, A. A. 1910. The Nevada lupines III. *Muhlenbergia* 6: 13-24. The Nevada lupines IV. *Ibid.* 6: 25-32. The Nevada lupines V. *Ibid.* 6: 97-108. The Nevada lupines VI. *Ibid.* 6: 109-113.
- . 1911. The North American lupines V. *Ibid.* 7: 85-95.
- Lindley, J. 1829. Half-shrubby lupine. *Bot. Reg.* 15: 1230.
- Piper, C. V. 1906. Flora of the state of Washington. *Contr. U. S. Nat. Herb.* 11: 350-358.
- Rydberg, P. A. 1917. Flora of the Rocky Mountains and adjacent plains, pp. 457-460.

# A BIOCHEMICAL STUDY OF THE INSOLUBLE PECTIC SUBSTANCES IN VEGETABLES<sup>1</sup>

CARL M. CONRAD

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## INTRODUCTION

A number of recent investigations on the chemistry of the pectic substances have renewed an interest in these plant constituents. Also their importance in various physiological processes has been emphasized lately by several investigators. However, very little progress has been made in our knowledge of these materials in vegetables. This paper deals chiefly with the extraction, quantitative determination, and occurrence of the insoluble pectic substances in typical vegetables and some fruits. There is considerable confusion in the nomenclature and characterization of the pectic substances. Three insoluble substances are here recognized and designated as: protopectin, pectic acid, and the insoluble pectates.

## HISTORICAL ACCOUNT

Fremy (9) first called the insoluble pectic substance, which during the ripening of fruits gives rise to pectin, "pectose." Much later Tschirch (15) called it "protopectin." The theory that protopectin is some sort of calcium compound was held first by Fremy. On the other hand, it has been considered by Mangin (11) to be a compound with cellulose. Fellenberg (7) claims to have proven conclusively that protopectin is not calcium pectate. Finally, Smolenski (12) suggests that protopectin may be a compound between pectin and a pentosan.

Fremy found that by cooking fruit he obtained much larger quantities of pectin than could be obtained by cold water alone and considered that this was due to the transformation of protopectin into pectin under the influence of acid. He produced pectin artificially by heating the insoluble fruit residues with dilute organic or mineral acids. Similarly, Chodnew (4) obtained a pectin from beet marc by heating with dilute hydrochloric acid. He called it 'pectinige acid' and thought it might be the same as Fremy obtained from fruit although it had a slightly acid reaction. That protopectin is not decomposed into pectin or pectic acid by alkali has been shown recently by Farnell (6) and Hardy (10).

Fremy states that no means is known for isolating pectose (protopectin) in pure form, and Fellenberg much later corroborates this statement.

<sup>1</sup> Published with the approval of the director, Maryland Agricultural Experiment Station.

However, Sucharipa (14) claims to have dissolved away a part of the cellulose of the cell walls of orange peel with copper-oxid-ammonia and obtained a residue of pure protopectin. When the protopectin was hydrolyzed with ammonium oxalate solution some cellulose was released, and he thought that this existed in chemical combination with pectin.

Pectic acid was first extracted from plant tissues by Braconnot (1). He noted its acid properties, its ready solubility in alkali, and its ability to form an abundant jelly when precipitated with hydrochloric acid. He also prepared the ammonium and potassium salts and determined the ratio of potash to acid to be 15 parts of the former to 85 parts of the latter. Other salts have been prepared by Fremy, Chodnew, and Fellenberg. Only the ammonium and alkali salts are soluble in water. Fremy discovered that pectic acid and the pectates are soluble in the neutral solutions of ammonium oxalate, tartrate, and citrate. Mangin held that these form double salts with pectic acid.

Fellenberg (7) showed that pectic acid differs from pectin only by a  $\text{CH}_2$  group for each carboxyl group present. This results from the fact that the group  $\text{COOCH}_3$  in pectin becomes  $\text{COOH}$  in pectic acid. He postulates eight carboxyl groups in pectic acid. In pectins these are more or less methylated, the more carboxyl groups methylated the more soluble being the resulting pectin.

Ehrlich (5) demonstrated the presence in the pectic acid molecule of galacturonic acid and galactose. According to Fellenberg's (8) later work, pectic acid contains two moles of arabinose, one mole of methyl pentose, one mole of galactose, and eight moles of galacturonic acid, the latter containing the carboxyl groups. Sucharipa has suggested that pectic acid may be combined with cellulose through the carboxyl groups and that the remaining carboxyl groups may be methylated. That pectic acid is the basal molecule of the pectic substances seems now well established.

#### EXPERIMENTAL

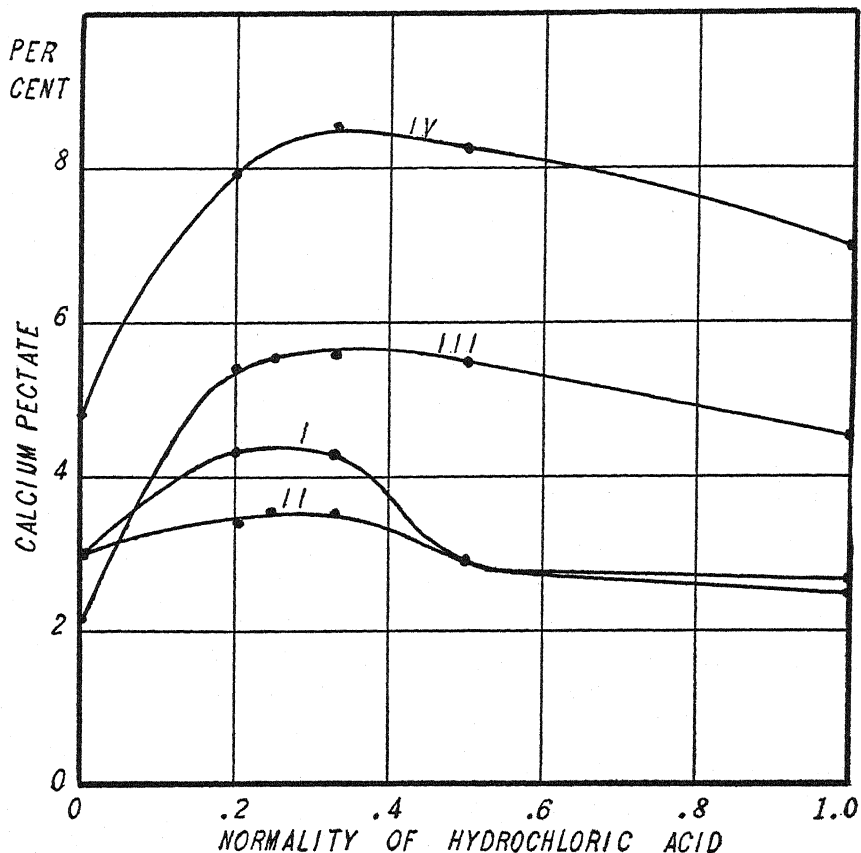
##### The Application to Vegetables of the Carre Method for Protopectin

The only method for the quantitative determination of protopectin which has been proposed up to the present is that of Carre (2). According to her method for apples, the finely ground material is covered with 100 cc. of *N*/20 hydrochloric acid, placed in an autoclave, and heated at  $110^\circ \text{C}$ . for one hour.<sup>2</sup> This process hydrolyzes the protopectin and converts it into pectin. The latter is washed out of the residue and determined by the Carre and Haynes (3) method for soluble pectin, namely, by converting it into calcium pectate and weighing the latter.

In attempting to adapt the Carre method to vegetable tissues it was first necessary to know whether this concentration of acid, the temperature, and time of heating would give the best results. Consequently these

<sup>2</sup> Miss Carre has recently modified her method, using *N*/100 HCl and boiling at atmos-

conditions were systematically varied and applied to different vegetable material. The vegetables used are very appropriate material since they contain no water-soluble pectin. Some potato pulp was washed thoroughly with water and dried. Samples were then heated under the conditions of the Carre method except with different concentrations of hydrochloric acid, and the amounts of hydrolyzed protopectin were determined. Next

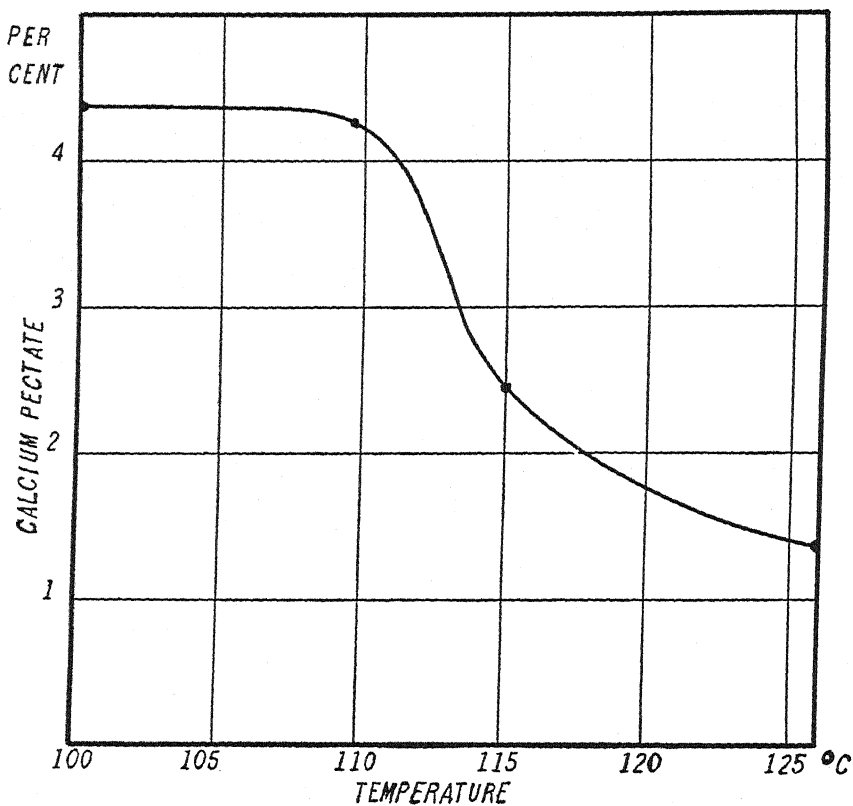


TEXT FIG. 1. Hydrolysis of protopectin by heating one hour with different concentrations of acid at the temperatures indicated. Curve I, potato ( $110^{\circ}\text{C}.$ ); curve II, potato ( $100^{\circ}\text{C}.$ ); curve III, onion ( $100^{\circ}\text{C}.$ ); curve IV, parsnip ( $100^{\circ}\text{C}.$ ).

the acidity which gave the highest yield was used, but the temperature was varied. With acidity and temperature fixed, the period of heating was varied. Finally, using potatoes, parsnips, and onions, the effects of different acid concentrations, under the conditions of temperature and time of heating already found most suitable, were determined. The results obtained in this series of experiments are shown in tables 1-4, and in figures 1-3. The protopectin is calculated in terms of calcium pectate in the air-dry vegetable.

TABLE 1. *Amounts of Protopectin Obtained by Using Different Concentrations of Acid*

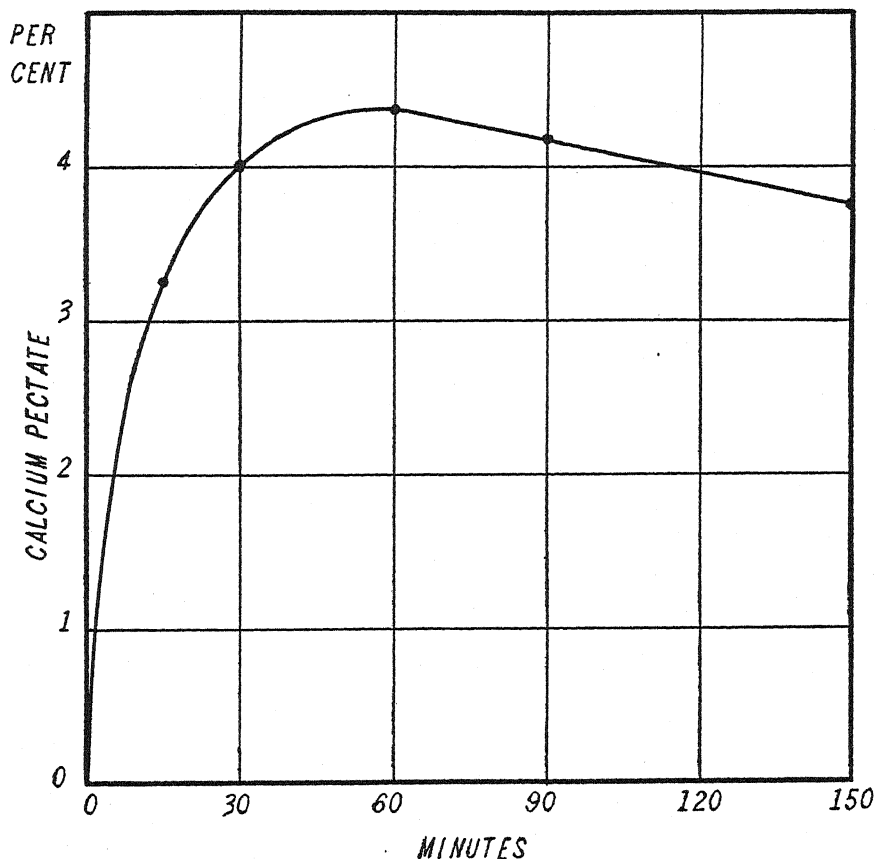
Potato	Distilled Water	Concentration of Hydrochloric Acid			
		N/50	N/30	N/20	N/10
Calcium pectate percent . . . . .	3.11 2.71	4.37 4.29	4.33 4.28	2.39 2.84	2.42 2.49

TEXT FIG. 2. Hydrolysis of protopectin as influenced by temperature above boiling. Samples of Irish potatoes were heated with 0.033 *N* (N/30) hydrochloric acid for one hour at the different temperatures.TABLE 2. *Effect of Varying the Temperature, Acid of N/30 Concentration being Used in All Cases*

Potato	Temperature of Heating in Autoclave, ° C.			
	Boiling (100)	109.5	115.0	126.0
Calcium pectate percent . . . . .	4.46 4.27	4.26 4.28	2.47 2.40	1.97 .69

TABLE 3. *Effect of Varying Time of Heating with N/30 Acid at Boiling Temperature*

Potato	Time of Boiling, Minutes				
	15	30	60	90	150
Calcium pectate percent.....	3.18 3.32	3.97 4.01	4.46 4.27	4.18 4.15	3.93 3.55



TEXT FIG. 3. Hydrolysis of protopectin in relation to period of heating. Samples of Irish potatoes were heated with 0.033 *N* (*N*/30) hydrochloric acid at boiling temperature (100° C.) for different periods of time.

In carrying out the analyses whose results are given in tables 1-4, several difficulties were encountered. One of these was the filtering of the viscous starch solutions obtained, particularly in the case of potatoes. Trials were made with solutions of malt and takadiastase, but, while these reduce the viscosity, they also destroy the pectin. Finally, this difficulty was completely solved by the use of saliva, which quickly reduces the

viscosity and does not attack the pectin. Difficulty in filtering was also encountered in the case of onions. This was due not to starch but to some other constituent contained in the material. This difficulty was fairly well solved by a preliminary extraction of the onion material with alcohol until most of the yellow coloring matter was removed. The proteins in potatoes also gave trouble as a certain amount of them were carried down with the calcium pectate precipitate and could not be removed by washing. This contamination did not occur to an appreciable extent with either parsnips or onions. It is a rather difficult matter to get rid of these proteins because the ordinary metal protein precipitants also precipitate the pectin. Some work with trichloroacetic acid indicated that this reagent might be effective in removing the protein. However, because of the unknown action of such reagents on the pectin, it was believed better to develop the method first on materials in which the proteins did not interfere. In the case of potato the proteins were estimated by the Kjeldahl method and subtracted from the weight of calcium pectate.

TABLE 4. *Effect of Varying the Acidity at Boiling Temperature for One Hour*

Concentration of Hydrochloric Acid	Calcium Pectate Percent		
	Potato	Parsnip	Onion
Distilled water.....	....	4.82	2.15
N/50.....	3.39	7.95	5.39
N/40.....	3.47	9.11	5.53
N/30.....	3.48	8.53	5.60
N/20.....	2.85	8.22	5.49
N/10.....	2.67	6.99	4.55

In the method used by Carre for determining the protopectin in apples, the pectin, after hydrolysis, was quantitatively washed from the disintegrated tissues. However, in all the determinations here given the hydrolyzed mixture was simply washed into a volumetric flask, neutralized, and made to volume. This digression from the Carre method was justified by results of experiments to compare the two methods of procedure.

Returning now to the tables and figures, we see that varying the acidity, temperature, and time of heating causes a great variation in the percentages of protopectin obtained. By varying the acid concentration in the Carre method it was found that N/30 to N/50 concentration gave the greatest amount of protopectin. These same concentrations of acid also gave the best results at boiling temperature. The higher temperatures began to be destructive to the liberated pectin at slightly above 110° C. The destruction of the pectin by high temperatures has also been shown recently by Hardy (10) and Farnell (6). Finally, a period of heating at boiling temperature with N/30 acid longer than one hour destroyed some of the liberated pectin.

The cause of the great variability in amounts of protopectin found with different concentrations of acid merits further consideration. Is it due to the fact that the pectin liberated is attacked by the dilute acid and partially decomposed so that it is not estimated by the Carre and Haynes method? This might account for the smaller amounts of protopectin obtained with the more concentrated acid solutions. It would also account for the decreased amounts obtained with longer periods of heating or at higher temperatures. In order to throw some light upon this question, an experiment was carried out on apple pectin. Aliquot portions of a solution of commercial apple pectin (Certo) were heated for an hour with different concentrations of acid. The solutions were then filtered and the pectin in the filtrate was determined. The percentages of calcium pectate thus obtained, based on the solids in the commercial product, are shown in table 5.

TABLE 5. *Destruction of Apple Pectin by Heating with Different Acid Concentrations*

Apple Pectin	Concentration of Hydrochloric Acid		
	N/30	N/20	N/10
Calcium pectate percent.....	20.42 20.42	19.69 20.28	18.02 17.66

An inspection of table 5 shows that increasing concentration of acid has a destructive effect upon apple pectin. The effect, barely appreciable for N/20, becomes very pronounced at N/10. It would seem, therefore, that the decrease in protopectin obtained with potatoes, parsnips, and onions by increasing acid concentration was due to the destruction of the pectin by the stronger acid. These experiments accordingly indicate that N/20 concentration of hydrochloric acid in the Carre method is slightly too concentrated for hydrolysis of the protopectin in these vegetables.

The foregoing experiments therefore indicate that the best procedure for the acid hydrolysis of protopectin in vegetables is to boil with N/30 to N/40 hydrochloric acid at atmospheric pressure for one hour. Longer periods of boiling, or higher temperatures, or greater concentrations of acid, all lead to destruction of a part of the pectin which has been liberated. These conditions will probably be applicable to all vegetable materials which contain only protopectin. However, we shall now show the limitations of these statements when applied to certain materials which contain other insoluble pectic substances.

#### Determination of Protopectin in the Presence of Pectic Acid or its Salts

Many of the earlier workers held that pectic acid and calcium pectate occur in plant tissues. Mangin (11) particularly developed this idea, and



arried out many experiments which seemed to show that the middle lamella of most soft parenchymatous tissues consists of pectic acid and pectates, chiefly calcium pectate. Mangin used solutions of ammonium oxalate, citrate, or tartrate as a solvent for pectic acid and the pectates. These solutions apparently form a soluble double salt with pectic acid whether it is present in the free state or combined with a metal.

In the work which follows no attempt will be made to separate pectic acid from its salts, since all are extracted alike by the ammonium salts used. Sodium hydroxid solutions, which have been used by microchemists to distinguish pectic acid from the pectates, were found by experiments to decompose calcium pectates and to dissolve a considerable part of the pectic acid radical. Therefore, the term pectic acid as used in this connection will include any of its salts that may be present in the plant material.

The application of solutions of ammonium oxalate and citrate to dried potato, parsnip, and onion tissues showed at once the presence of large quantities of pectic acid. The ground material was simply stirred with a one-percent cold solution and the filtrate was treated with a small amount of concentrated HCl. The separation of a voluminous gel showed the presence of pectic acid. Tests with cold solutions of ammonium oxalate and citrate on material which contained no pectic acid showed that the protopectin was unaltered by these solutions.

After this demonstration of comparatively large amounts of pectic acid in certain vegetable material, it became of interest to investigate the effect of its presence on the protopectin determination. In one experiment, the residues from a protopectin determination on parsnips were used. These were covered with one-percent ammonium oxalate solution and heated at the boiling point for one hour. After being allowed to cool, the extracts were filtered and the residues were washed. The pectic acid extracted by the ammonium oxalate was converted into calcium pectate by the following procedure: Sufficient concentrated hydrochloric acid to give a one-percent solution was added to the filtrate to precipitate the pectic acid. The pectic acid was filtered off and washed to remove the oxalate ion. It was then dissolved through the filter into a clean beaker with 100 cc. *N/10* sodium hydroxid. The filtrate was diluted to 400 cc. and treated from this point on as in the Carre and Haynes method for soluble pectin. The amounts of calcium pectate thus obtained are shown in table 6.

TABLE 6. *Pectic Material Extracted by One-percent Ammonium Oxalate from Parsnips after the Removal of Protopectin*

Sample	Calcium Pectate	
	Actual Weight	Percent
1.....	0.0276	2.76
2.....	0.0262	2.62

This experiment shows that pectic acid remained in the parsnip material after the protopectin determination and could be extracted by ammonium oxalate solution.

In order to determine whether any pectic acid is dissolved by hot  $N/30$  HCl, some samples of pure calcium pectate were subjected to the same treatment as was used for protopectin, *i.e.*, heated with  $N/30$  HCl for an hour and filtered. The filtrate was treated according to the Carre and Haynes method for pectin except that alkali was not added for saponification. In two instances the insoluble residue of calcium pectate was washed from the filter, dried, and weighed. The amounts of calcium pectate recovered from the extracts together with the residues are shown in table 7.

TABLE 7. *Partial Solution of Calcium Pectate by Boiling 0.1 G. Samples with  $N/30$  HCl for One Hour*

Source of Calcium Pectate	Calcium Pectate from Extract		Residue after Treatment	
	Actual Weight	Percent	Actual Weight	Percent
Peach pectin.....	0.0133	13.3	0.0610	61.0
Peach pectin.....	.0099	9.9		
Peach pectin.....	.0121	12.1		
Onion.....	.0170	17.0	0.0512	51.2
Onion.....	.0175	17.5		
Onion.....	.0098	9.8		

It is shown in table 7 that  $N/30$  HCl will dissolve a considerable amount of the pectate. The residue shows a greater loss than can be accounted for by the calcium pectate recovered. A part of this difference no doubt results from loss of calcium from the residue. It is very evident from the two foregoing experiments that the Carre method for the determination of protopectin in a plant material which contains pectic acid will give erroneous results depending upon the amount of the latter dissolved by the  $N/30$  acid treatment.

The next attempt was the quantitative removal and determination of pectic acid preliminary to the protopectin determination. Samples of dried parsnips and onions were ground to pass a 40-mesh sieve and extracted alternately with cold one-percent ammonium oxalate for several hours and boiling  $N/30$  hydrochloric acid for one hour. The order of extraction and the results are shown in table 8.

TABLE 8. *Insoluble Pectic Compounds Removed from Onions and Parsnips by Alternate Extractions with Ammonium Oxalate and Hydrochloric Acid (Determined as Calcium Pectate)*

Order of Extraction	Calcium Pectate Percent	
	Onion	Parsnip
1-percent ammonium oxalate (pectic acid).....	2.33	4.77
Second extraction with ammonium oxalate.....	0.00	0.00
$N/30$ hydrochloric acid (protopectin).....	1.21	2.09
Third extraction with ammonium oxalate (pectic acid).....	3.63	3.14

According to table 8, the second extraction with ammonium oxalate did not remove any more pectic acid. However, after removing the protopectin with  $N/30$  HCl, ammonium oxalate again extracted abundant pectic acid. The reason for this result is not evident at present. Possibly the pectic acid obtained by the final extraction was held from the earlier extraction by impermeable walls composed partly of cellulose, partly of protopectin. On the other hand, it may be possible that the pectic acid obtained in this last extraction was chemically united in some way and was only released by the treatment for protopectin. However, this experiment shows that pectic acid can not be dissolved completely with ammonium oxalate until after the protopectin has been removed. This means that neither protopectin nor pectic acid can be completely removed from the vegetable material without affecting the other constituent.

### Combined Determination of Protopectin and Pectic Acid

An attempt was now made to overcome the objections to the removal of either of the insoluble pectic substances separately by developing a method which extracts both constituents simultaneously. In making the ammonium oxalate extraction with material containing protopectin, it has previously been necessary to use cold solutions since hot solutions readily hydrolyze protopectin. However, in combining the extraction of protopectin and pectic acid it should be possible to use a hot solution of ammonium oxalate, since in this case the hydrolysis of protopectin is desired. Furthermore, a hot solvent may be expected to be much more efficient as an extracting agent than a cold one.

The first combined solvent tried was composed of a one-percent solution of ammonium oxalate in  $N/30$  hydrochloric acid. Samples of parsnip were heated with this solution for one hour under a reflux condenser. The mixtures were cooled, filtered, and the residues were washed thoroughly with water. After neutralizing the filtrates with normal sodium hydroxid the pectic acid was precipitated with one-percent hydrochloric acid. The filtrate from the pectic acid was first neutralized and then treated with sodium hydroxid to saponify the pectin. After standing for 24 hours the resulting pectic acid was precipitated with one-percent hydrochloric acid and converted into calcium pectate. At the same time some samples of parsnips were extracted as described above, and the filtrates were subjected at once to alkali hydrolysis without attempting to remove pectic acid. The total amount of pectic material thus obtained is to be compared with the sum of the protopectin and pectic acid, determined separately (table 9).

TABLE 9. *Quantitative Separation of the Insoluble Pectic Materials in Parsnips by the Method of Combined Extraction (Determined Separately and Combined as Calcium Pectate)*

	Pectic Substance	Calcium Pectate Percent
Pectic acid.....	8.02	
Protopectin.....	1.75	
		0.71

This experiment shows that the combined method of extraction removes both protopectin and pectic acid and that they may be determined either separately or together since the total calcium pectate was approximately the same in both cases. It is also interesting to note that a large part of the pectic material is in the form of pectic acid.

In the experiments heretofore described the pectic acid has been precipitated with one-percent hydrochloric acid. While precipitation by this method seems to be fairly complete, still the procedure is open to serious objections. The strong acid may chemically alter the pectic acid, as contended recently by Wichmann (16). Also, the washing necessary to remove the oxalate ion dissolves a certain amount of pectic acid. These difficulties and objections were happily met through the use of ammonium citrate solution in place of ammonium oxalate.

Ammonium citrate is just as efficient a solvent for pectic acid and calcium pectate as is ammonium oxalate. In addition it has the property of not giving a precipitate with cold solutions of calcium chlorid, although when the solutions are boiled a precipitate of calcium citrate separates out immediately. These facts permit of an important improvement in the procedure for the combined determination of protopectin and pectic acid.

The following experiment was planned to determine, after hydrolysis of protopectin, the time necessary for complete extraction of pectic acid with ammonium citrate. Samples of the same parsnips used in the previous experiments were heated for one hour with *N*/30 hydrochloric acid. The mixture was then neutralized with sodium hydroxid and sufficient concentrated ammonium citrate solution was added to make a one-percent solution. It was again boiled for different periods and filtered, and the residues were washed with hot water. The filtrate and washings were made to mark in a volumetric flask. One aliquot was treated directly with acetic acid and calcium chlorid to precipitate the pectic acid. Another aliquot was treated with sodium hydroxid for a period of about 24 hours to saponify the pectin. The total pectic acid was then precipitated with acetic acid and calcium chlorid. The calcium pectates were filtered off cold and thoroughly washed in order to remove the citrate ion, which would otherwise be precipitated as calcium citrate in the subsequent heating. It was found best to wash the pectic acid fraction first with a one-percent solution of calcium chlorid. It is thought that this fraction still retains a certain amount of methoxyl groups which make the gel tend to dissolve and thereby clog the filter. However, no difficulty was experienced when the gel was first washed with 0.5% calcium chlorid and then finally with water. The gel from the pectin fraction was washed with water only. The calcium pectate was next washed back into the original beaker and boiled several minutes with a considerable volume of distilled water to extract any remaining soluble salts. The gel was filtered hot, washed until free from chlorids, and finally dried in a small beaker. The percentages based on

dry weight of parsnip are given in table 10. Protopectin is calculated by the difference between the combined protopectin and pectic acid and the pectic acid determinations.

TABLE 10. *Protopectin and Pectic Acid Obtained from Parsnips by Varying the Time of Heating with Ammonium Citrate*

Boiled 1 Hr. with N/30 HCl, then with Ammonium Cit- rate (Minutes)	Pectic Materials as Calcium Pectate Percent		
	Protopectin and Pectic Acid	Pectic Acid Only	Protopectin (by Difference)
15.....	9.60	6.79	2.81
30.....	9.92	7.24	2.68
70.....	9.40	7.04	2.36

This table shows that 15 minutes' boiling with ammonium citrate solution is not sufficient to give the maximum amount of total pectic material (protopectin and pectic acid). This is obtained by boiling for 30 minutes. It also shows that a slow loss of pectin occurs even when it is boiled in neutral solutions.

In view of the fact that long boiling destroys the pectin, it seems advisable to make the heating period as short as possible. By reference to table 3, it will be noted that almost the maximum amount of pectin was obtained after boiling for 30 minutes. If, now, the heating with acid were stopped, the acid neutralized, ammonium citrate solution added, and the boiling again continued for 30 minutes, the total time of heating could be considerably shortened without shortening the time of heating with the citrate. Accordingly, an experiment designed to test this method was carried out. The results are shown in table 11. The parsnips were not the same as those used for table 10, so the figures of the two tables can not be compared. The sugar beets were prepared by drying thin slices in a vacuum oven at 70° C.

TABLE 11. *Comparison of 30-minute and 60-minute Periods of Hydrolysis in Combined Method; Figures Represent Percentages of Calcium Pectate*

Material	Hydrolyzed 30 Minutes			Hydrolyzed 60 Minutes		
	Protopectin	Pectic Acid	Total	Protopectin	Pectic Acid	Total
Parsnips.....	1.44	9.10	10.54	1.62	8.55	10.17
Sugar beets.....	4.82	.00	4.82	4.77	.00	4.77

A second treatment of the material exactly like the first did not yield a trace of either pectin or pectic acid. Therefore, it is evident that a 30-minute period of hydrolysis with N/30 HCl followed by extraction with

boiling one-percent ammonium citrate for 30 minutes extracts all the insoluble pectic material from these tissues.

Finally it seemed desirable to compare the suggested combined method of extracting with the modified Carre method for protopectin. Both peaches and parsnips were used for the experiment. These were prepared from the fresh material by drying according to the Spoehr method (13). The results are presented in table 12.

TABLE 12. *Comparison of the Modified Carre Method with Combined Method of Extraction and Determination; Percentages of Pectic Material Based on Dry Weight*

Material	Modified Carre Method	Combined Method		
	Protopectin	Protopectin	Pectic Acid	Total
Peach.....	5.11	5.14	0.00	5.14
Peach.....	5.12	5.10	.00	5.10
Parsnip 1.....	9.34	5.76	7.88	13.64
Parsnip 2.....	9.05	4.91	5.93	10.84

An inspection of the table shows that for peaches, which contain no pectic acid, almost exactly the same amount of protopectin was obtained by both methods. In parsnips we find a different situation. The pectic acid is only partially extracted by the Carre method. This is shown by the fact that considerably larger amounts of total pectic material were obtained by the combined method. The two sets of analyses for parsnips must not be compared since they are not duplicates. From this experiment we may draw the conclusion that the modified Carre method for protopectin will not give dependable results when pectic acid is present.

#### Effect of Alcohol Treatment and Different Methods of Drying on the Amounts of Pectic Acid Found in Parsnips

A satisfactory method having been worked out for the simultaneous removal and determination of the two insoluble pectic substances, it seemed worth while now to compare different methods of preparation of the material for analyses in regard to the relative amounts of pectic acid and protopectin.

A number of parsnip roots were cleaned and cut into small pieces. The pieces were thoroughly mixed and 25-gram samples were weighed out. Sample 1 was dried over night at 98° C. in a drying oven with good air circulation. During the first hour the material was heated in its own steam according to the method of Spoehr (13). Sample 2 was dried for 24 hours in a vacuum oven at 70° C. and 25 inches of vacuum. Sample 3 was treated immediately with boiling 95 percent alcohol and boiled 10 minutes. The alcohol was filtered off and the material was dried at 80° C. Sample 4 was dried at room temperature and in the sun. The dried samples were ground to pass a 40-mesh sieve, and the protopectin and pectic acid

were determined by the combined method already described. The results are shown in table 13.

TABLE 13. *Comparison of Treatments of Parsnip Tissue for Pectic Analysis; Figures Based on Dry Weight and in Terms of Calcium Pectate*

Sample No.	Treatment of Tissue	Protopectin Percent	Pectic Acid Percent
1	Dried at 98° in air oven.....	6.84	3.95
2	Dried at 70° in vacuum.....	3.83	9.61
3	Boiling alcohol.....	10.69	0.00
4	Sun dried.....	7.36	5.26

This table shows that protopectin was obtained in all cases. However, where boiling alcohol was used no pectic acid was found. In all other cases pectic acid was found in considerable quantity. This experiment, therefore, discloses the very important fact that the pectic acid previously found in parsnips probably does not exist there at all but was produced from protopectin during the preparation of the tissues for analyses.

An experiment similar to the preceding was also carried out with potatoes. Samples prepared by drying always contained pectic acid, while none whatever was found when the material was prepared by the alcohol method. The possibility that pectic acid originally present might have become esterified was tested by boiling pectic acid with ethyl alcohol, both under the conditions used for the tissues and for longer periods, but in neither case was a detectable amount of soluble pectin produced.

These experiments show conclusively, therefore, that pectic acid may be produced as a secondary product in the preparation of the sample. What is the cause of this transformation? An inspection of the table shows at once that it is not due to high temperature. For instance, sample 3, which was first boiled with 95 percent alcohol and then heated at 80° C. until dry, gave no pectic acid, while sample 2, dried at a lower temperature, gave almost 75 percent of its pectic material in this form. Similarly, sample 4, dried at a very low temperature gave over 40 percent of the pectic material as pectic acid. The experiment indicates rather the presence of enzymes which split off methyl alcohol and leave behind the pectic acid radical. Thus the treatment of sample 3 is such as almost instantly to check enzyme action. On the other hand, the 70° C. used for sample 2 would probably only slowly destroy enzymes after having first accelerated their action. Likewise the figures for samples 1 and 4 would indicate that enzyme action had been soon checked or that it was much slower.

If pectic acid is produced by enzyme action during the process of drying, then the method of preparation of the sample is very important when information is desired as to the natural occurrence of pectic acid or pectates. If the latter are to be sought in the material, it should be prepared by a method which eliminates enzyme action.

### Amounts of Protopectin and Pectates in Various Plant Tissues

It is the general opinion that pectic acid and pectates, particularly calcium pectate, occur to a considerable extent in plant tissues. In view of the discovery that pectic acid may be rapidly produced by enzym action in certain tissues during drying, a survey was made of the nature of the insoluble pectic materials in a number of vegetables and fruits, employing the alcohol method of preparing the tissues for analysis.

The material for these analyses was obtained by slicing a previously weighed amount of the fresh material (10–25 grams) into 100 cc. of boiling 95% ethyl alcohol. The slices were cut about 1 to 3 mm. thick so that the hot alcohol could quickly penetrate them. They were boiled in the alcohol for 10 minutes, and then allowed to stand until analysis could be made. The alcohol was filtered off and the material was dried on a filter in a vacuum oven at 80° C. and 25 inches of vacuum. The dried material was ground to pass a 40-mesh sieve, and protopectin and pectic acid were determined by the combined method. The results thus obtained are given in table 14.

TABLE 14. *Protopectin (Including Pectin) and Pectic Acid in Different Plant Tissues; Analyses Based on Dry Weight*

Tissue	Protopectin and Pectin Percent	Pectic Acid Percent
Strawberries, green.....	8.82	0.00
Strawberries, ripe.....	6.16	0.00
Tomatoes, green.....	5.45	0.00
Tomatoes, ripe.....	2.92	0.00
Potato.....	2.00	0.00
Beet, red.....	3.82	0.00
Carrot.....	10.04	0.00
Turnip.....	11.93	0.00
Radish, pithy.....	26.87	15.37
Parsnips.....	10.68	0.00
Orange.....	0.10*	trace
Bananas.....	2.24	0.00
Cherries, green.....	11.42	0.00
Cherries, ripe.....	4.32	0.00

\* Based on wet weight.

From this table it appears that generally vegetable and fruit tissues do not contain pectic acid or pectates. Only in one case, that of very pithy radishes, was an appreciable amount of pectic acid found. The pectic acid in this case, however, was unmistakable, and the total amount of pectic material was extremely large in comparison to the other tissues. Very likely the trace of pectic acid found in orange came from the very rapid transformation of the soluble pectin in the juice by pectase, since the



macerated tissue of orange has been observed to set to a gel in the course of several minutes.

#### SUMMARY AND CONCLUSIONS

1. A study was made of the insoluble pectic constituents, chiefly in vegetables.

2. A detailed study was made of the Carre method for the quantitative determination of protopectin in fruits, with the view of standardizing it for use with vegetables. Modifications of acidity of hydrolyzing solution and temperature have been introduced which make the method applicable except in cases in which pectic acid or pectates are present.

3. When both protopectin and pectic acid are present in a vegetable tissue, neither can be removed separately, but both can be removed simultaneously and the resulting pectin and pectic acid separated afterwards.

4. A method has been described for the simultaneous removal and quantitative separation and determination of protopectin and pectic acid or its salts.

5. The true nature of the pectic substances in vegetable and fruit tissue was not revealed when the samples were dried, since all the different methods used for drying converted protopectin into pectic acid. This transformation was prevented by slicing the material into boiling alcohol. Many cases of pectates reported in plant tissues are probably the result of enzym action during the preparation of the material for analysis.

6. Finally, a comparison was made of the amounts of pectic constituents in a variety of plant tissues, employing the alcohol treatment and method of combined extraction suggested in this paper. Protopectin, or its hydrolytic product pectin, was found in all tissues studied, but pectic acid or pectates were found only in radishes.

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# GROWTH OF TOMATO CUTTINGS IN RELATION TO STORED CARBOHYDRATE AND NITROGENOUS COMPOUNDS

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## INTRODUCTION

The factors which affect the character of the growth responses in plants constitute a subject of general interest. Current literature bearing on the topic indicates that the chief determining influences may be of a chemical nature.

Sachs (22) assumed that the plant produces special growth-promoting substances in very small quantities and of as many sorts as there are kinds of organs. "These flower-forming substances," he says, "act, like ferments, upon large masses of plastic material, although they themselves are present in exceedingly small amounts."

In his earlier experiments on *Bryophyllum calycinum*, Loeb (13) attempted to explain, at least in part, the polar character of regeneration by assuming the presence of substances which act as growth-inhibitors within the plant. H. S. Reed (16) has suggested a similar hypothesis.

Loeb (12) held that the phenomena of regeneration may be explained on the basis of the supply of food materials alone; "that equal masses of stems produce, under equal conditions of illumination, temperature, etc., approximately equal masses of shoots and roots in equal time." A second physiological factor, evidently a substitute for the former conception of inhibitory substances, is also considered:

That the flow of sap in a leaf or stem is secondarily directed towards that part of a leaf or stem where the more rapid growth of shoots or roots occurs. This explains why only some but not all of the *Anlagen* for roots or shoots in a leaf or stem will persist in growing out; while the growth of others will stop.

He suggests (14) that the polar character of regeneration is due, not to any of the chemical differences between the ascending and descending sap, but to a difference in the nature of the tissues that are primarily reached by the ascending and descending sap. It is not clear whether he assumes the specific substances to be building materials or hormones.

Robertson (21) points out that the separation of the postulated two formative materials for roots or shoots is never quite complete; that there is a gradient in concentration of the root-forming substance, which increases from above downward. He suggests that

This mode of distribution under the action of gravity is inconceivable unless the root-forming substance is continually in process of manufacture. Obviously if the rate of manufacture were a little slower than the rate of settling by gravity, the result of drainage of the material from the apical end where it is undergoing manufacture would be to bring about just such a distribution or gradient as that which we have inferred.

We are thus led to suppose that the theory would be in a precarious position if the process of manufacture went on faster than the rate of settling by gravity. That variations in the gradients of root- and shoot-forming materials in the stem may occur along with differences in the chemical composition will be shown later in this paper. Robertson finds still more serious difficulties in attempting to apply this theory to the phenomena of regeneration of simple animals such as Planaria.

#### Interrelation between Growth and Materials outside the Plant

Much study has been made of the effect of mineral nutrients, particularly nitrogen, upon the relative amounts of root and shoot growth. Godlewski (7) suggested that, in the absence of nitrogen, root-formation is favored more than shoot-formation, on the assumption that a greater portion of the nitrogen of the substrate may be utilized if there is more extensive root-development. Chandler (1) found that root-growth is increased much less than top-growth by the addition of nitrates to peach trees. Gericke (6) has found that an abnormally large root-development of wheat seedlings in proportion to that of tops is primarily associated with a deficiency of nitrogen in the substrate.

#### Interrelation between Growth and Materials within the Plant

##### *Carbohydrates*

The significance of a large supply of carbohydrates as a factor in insuring an extensive development of roots has been discussed by a number of investigators. Dachnowski (3) grew tomato cuttings in a variety of solutions and found that cane sugar somewhat increased root-growth. Knudson (10) has recorded a marked influence of certain sugars on root-growth of seedlings, in relation to both their extent and their branching. Curtis (2) found that immature twigs of *Ligustrum ovalifolium* showed an increased root-development when grown in cane sugar solutions. Mature twigs of similar kind did not respond in the same way. Kraus and Kraybill (11) observed that

A decided reduction in the development of root systems of tomato plants accompanied a continued removal of leaves from the top. According to microchemical tests this practice also resulted in a marked decrease in the carbohydrates in the stems and a decided reduction in vegetative extension.

Ellis and Eyster (4) studied the effects of insulin and glucokinin on maize seedlings of green and non-green types. They found a retardation in growth of seedlings grown in distilled water containing 1 to 0.005 percent

of insulin or glucokinin. In the stronger solutions, the formation of secondary roots was practically inhibited; in those weaker than 0.005 percent, growth was somewhat increased.

### *Nitrogen*

Nitrogen stored within the plant may be used in new tissue-growth when nitrogen is lacking in the nutrient medium. Zaleski (26) found that synthesis of protein occurs in sprouting bulbs, tubers, and roots without any absorption of nitrogen from without and without any increase in nitrogen. Roberts (20) has shown that apple trees can take in nitrogen, store it as a reserve, and later utilize it in growth as well as create and utilize a carbohydrate reserve. Nightingale (15) found a marked increase in vegetativeness when he transferred tomato plants grown in quartz sand in a solution lacking nitrates from a 14- to a 6-hour day. Chemical analyses of the plants showed that the total amount of nitrogen in proportion to the carbohydrates had notably increased, and that the increase in nitrogen was particularly in the water-soluble fraction. Kraus and Kraybill (11) incidentally observed that cuttings high in carbohydrates but low in nitrogen produced chiefly roots; cuttings having intermediate proportions of carbohydrates to nitrogen produced both roots and shoots, whereas highly vegetative cuttings produced neither roots nor shoots.

### **Interrelations of Materials within and without the Plant**

Exact knowledge concerning the causative factors that influence the growth responses cannot be gained by studying either external or internal conditions alone. The utilization of food reserves may be influenced by such factors as light, temperature, and nutrient medium. Indeed, it is probably true that these external factors produce their effects largely through modifications of the available foods. Nightingale's results (15) afford some evidence in support of the idea that light exerts its influence in growth through its effect upon the nutritive materials within the plant. Klebs (9) maintained that no plants have the capacity for development unless they possess certain proportions of carbohydrates and nutrient salts. Some of the relations of carbohydrates to nitrogen have been suggested by Fischer (5) and Kraus and Kraybill (11).

The effects of different proportions of carbohydrates and nitrogen with respect to root- and shoot-growth have been studied by Robbins (19) and Turner (25). Robbins found that excised roots of several kinds of seedlings would, under sterile conditions, develop a considerable root system in a mineral nutrient solution containing carbohydrates, but that little growth occurred in cultures to which no nitrate was added; but the roots would not continue to grow indefinitely under either of these conditions. He thus concluded that the root of a seedling derives some materials from the seed other than glucose, the mineral salts, water, and oxygen, which are

necessary for continued growth and which the root can not synthesize in the dark in solution cultures from which the materials are supplied.

Turner (25) studied the effect of nitrates upon the ratios of shoot- to root-growth in certain seedlings and states that

The increased ratio of tops to roots which results from increasing the amount of nitrate in the solution may be explained on the basis of the increased use of carbohydrates in the tops because the greater nitrogen supply makes for greater growth. This results in a decrease in the supply of carbohydrates for the roots, which may bring about an absolute or relative reduction of root growth.

In my own investigations (17, 18), I have found that the proportions of carbohydrates to nitrogen are of significance in influencing the ratios of shoots to roots in tomato cuttings and plants.

#### The Use of Stem Cuttings as a Method of Studying Growth Responses in Relation to the Available Food Reserves

The synthesis of new protein materials from carbohydrate and nitrogenous reserves has been studied in germinating seeds by a number of investigators. It has, however, been difficult to tell whether or not these materials are being used in the same or in different proportions in producing the various organs. It seems that it should be possible, by using plant materials having extremely wide ranges of difference between their carbohydrate and nitrogenous constituents, to follow to some extent the utilization of varying proportions of these constituents in growth.

By varying the methods of culture, it is possible to secure tomato plants differing from the high ratios of 36.52 parts of carbohydrates (starch, sugar, free reducing substances) to one of nitrogenous matter, down to the relatively low ratio of 1.7 parts of carbohydrates to one of nitrogenous matter. For two reasons, the use of cuttings instead of entire plants offers distinct advantages in studying the utilization of food reserves during growth: (a) With isolated segments of the stem, taken from different levels of the plant, the substances stored at that level must be locally utilized, *i.e.*, in that special segment; (b) there is a wide difference in composition of cuttings, ranging from the most vegetative of all (*i.e.*, the top cuttings of the vegetative plants) to those highest in carbohydrates and lowest in nitrogenous content (*i.e.*, the basal cuttings of the high-carbohydrate plants). There is thus secured greater uniformity of individual pieces and yet wider variation between different types.

#### MATERIALS AND METHODS

The tomato plants from which the cuttings used in these experiments were obtained were of two general types. Those high in carbohydrates and low in nitrogen content were produced by first growing the plants to a height of 5 to 7 inches in small pots containing rich soil and then trans-

ferring them, after having washed all the organic matter from the roots, to 10-inch pots containing quartz sand to which was added a solution containing all the essential nutrient elements except nitrogen. The soil was kept moist by the addition of distilled water. Those plants very high in their nitrogenous content and relatively low in carbohydrates were produced by being grown continuously in a soil rich in nitrogen.

From 4 to 6 weeks were required for the first group of plants to become high in carbohydrates. Their content was determined by microchemical tests for starch, proteins, and nitrates. When no positive tests for free nitrates were obtained by using diphenylamine sulfuric acid on cut sections of stems taken from various levels, the plants were considered ready for use. The total quantity of starch and proteins that accumulated during the time the plants were grown in quartz sand varied considerably in different experiments. Time of year seemed to have a marked influence in this respect. Plants grown from the middle of November to the middle of January usually developed slowly, and the storage of both starch and proteins was much limited in quantity.

In preparing the cuttings, the plants were cut off at the surface of the ground and defoliated, then cut into segments 4 to 9 inches in length. In some of the experiments basal, middle, and top cuttings were made. The formulae given below were used in the preparation of the solutions used for series V, VI, and VII. These solutions were found to yield the best results.

*Solution Containing Nitrates*

*A*

Magnesium sulfate.....2%  
Monobasic potassium phosphate.....2%  
Potassium nitrate.....2%

*B*

Calcium chlorid.....3%  
Calcium sulfate.....2%  
Calcium nitrate.....4%

*Solution Lacking Nitrates*

*A*

Magnesium sulfate.....2%  
Monobasic potassium phosphate.....2%  
Potassium chlorid.....1%

*B*

Calcium chlorid.....4%  
Calcium sulfate.....2%

In preparing the solution for the cuttings, 100 cc. of solution *A* were made up to one liter in distilled water and an equal quantity of solution *B* was made up in the same way. These diluted solutions of *A* and *B* were mixed at the time of using. The solutions with and without nitrates were both prepared in this manner. A few drops of a 1% solution of ferric citrate were added to each liter of the solution before using. In each experiment the nutrient solutions were renewed every 4 days.

In the first 5 series of experiments, accurate weight determinations were made of the cuttings themselves before and after regeneration and also of the roots and shoots produced. Records were taken for each individual. The cuttings and the roots and shoots produced were dried

separately *in vacuo* at 65° C. to constant weight and the dry weights were recorded.

The possibility of leaching of some of the food substances from the cutting into the solution was considered, and, in order to determine if it did occur to such an extent as to modify the growth response, the cut basal ends of the cuttings used in one experiment were coated with a mixture of beeswax and paraffin. No noticeable difference was found in the growth responses obtained, as compared with unsealed cuttings.

The chief objection to the methods used may perhaps be that the cuttings were grown in water cultures. In the first two series of experiments, duplicate tests were made by growing one lot of the cuttings in sand moistened with the same kinds of nutrient solutions as were used for the water-culture tests. The same general results were obtained with high-carbohydrate cuttings as were obtained with similar cuttings from the water cultures except that the total growth was somewhat less than in the latter. The vegetative cuttings did not grow at all well in sand. They appeared wilted, perhaps because their water requirements were higher than absorption could provide. These cuttings also were more susceptible to decay than were those grown in water cultures.

#### EXPERIMENTAL RESULTS

##### Series I: January 15 to February 5, 1921

The plants of the high-carbohydrate type used in this series were not so rich in carbohydrates as some used in later experiments. The cloudy weather of November and December, 1920, together with the short period of daylight, combined to make the plants somewhat more vegetative in their general appearance than those grown for later experiments. Three cuttings were made from each plant having longer stems and 2 from each of the shorter ones. In the case of stems that were cut into three segments, the middle ones were weighed together with the top segments. The cuttings from each plant were tied together with coarse thread and suspended from the meshes of a wire screen set over the top of a 2-liter battery jar. The lower ends of the stems were immersed to a depth of about 2 inches in the nutrient solution. The cuttings were placed in the light at a temperature of 18°–22° C.

The most conspicuous features of the growth responses may be stated as follows:

Cuttings high in carbohydrate content rooted much more profusely than did those low in carbohydrates regardless of the treatment applied. The former, when grown in the solution containing nitrogen, had a greater capacity for producing both roots and shoots than did the latter, but when grown in the solution without nitrogen they produced more roots but fewer shoots both as to number and weight than the vegetative cuttings. It thus appeared that:



1. A small supply of carbohydrates was associated with a relatively smaller quantity of roots than of shoots.

2. A small supply of nitrogen had a more limiting effect on shoot-production than it had on root-production.

### Series II: April 27 to May 20, 1921

This experiment was conducted according to the methods described for series I. In addition to the cuttings grown in light, a duplicate series was grown in darkness. Because of the difference in season, the temperature averaged somewhat higher (20° to 29° C., and for series I, 18° to 22° C.), the light was more intense, and the periods of illumination were longer than was the case with series I.

TABLE 1. *Series I, January 15–February 5, 1921; Average Green Weight of Roots and Shoots Produced by the Individual Cutting of Average Weight*

Type of Cutting	Solution	No. of Cuttings	Ave. Wt. of Cuttings		Ave. Wt. of Roots Produced per Cutting		Ave. Wt. of Shoots Produced per Cutting	
			Basal	Terminal	Basal	Terminal	Basal	Terminal
High carbohydrate.....	Plus nitrates...	23	5.177	5.136	0.666	0.300	1.134	0.921
	Minus nitrates.	19	5.108	5.298	0.611	0.192	0.100	0.110
Moderately vegetative.....	Plus nitrates...	16	6.794	7.020	0.049	0.007	0.518	0.320
	Minus nitrates.	15	7.165	7.279	0.144	0.018	0.586	0.577

TABLE 2. *Series I, January 15–February 5, 1921; Green Weight of Roots and Shoots Produced by 1 Gram of Original Green Material*

Type of Cutting	Solution	Green Wt. in G. of Roots Produced by 1 G. of Original Green Material		Green Wt. in G. of Shoots Produced by 1 G. of Original Green Material	
		Basal	Terminal	Basal	Terminal
High carbohydrate.....	Plus nitrates...	0.1287	0.0586	0.2190	0.1794
	Minus nitrates.	0.1196	0.0363	0.0196	0.0209
Moderately vegetative...	Plus nitrates...	0.0072	0.0010	0.0763	0.0456
	Minus nitrates.	0.0200	0.0025	0.0818	0.0792

The general types of growth responses in the light were similar to those which occurred with cuttings of series I, except that no shoots were produced by the basal high-carbohydrate cuttings grown in the solution lacking nitrates. The roots were shorter than those produced in series I, and it was supposed that this condition might be due to the somewhat higher temperature in which the cuttings of series II were grown. The quantitative results are indicated in tables 3 and 4.

TABLE 3. *Series II, April 27-May 20, 1921; Average Green Weight of Roots and Shoots Produced by the Individual Cutting of Average Weight*

Type of Cutting	Solution	Light or Darkness	No. of Cuttings	Ave. Wt. of Cuttings		Ave. Wt. of Roots per Cutting		Ave. Wt. of Shoots per Cutting	
				Basal	Terminal	Basal	Terminal	Basal	Terminal
High carbohydrate....	Plus nitrates...	Light....	20	2.504	2.338	0.609	0.376	0.990	0.945
		Darkness...	20	2.719	2.521	0.369	0.158	0.432	0.145
	Minus nitrates...	Light....	20	2.525	1.839	0.029	0.002	0.000	0.033
		Darkness...	20	2.455	2.249	0.094	0.021	0.000	0.004
Moderately vegetative.	Plus nitrates...	Light....	5	15.267	15.738	0.021	0.006	0.943	1.617
		Darkness...	4	18.330	18.057	0.006	0.000	0.000	0.116
	Minus nitrates...	Light....	5	15.088	13.530	0.019	0.035	0.541	0.632
		Darkness...	5	15.036	12.265	0.017	0.000	0.182	0.170

TABLE 4. *Series II, April 27-May 20, 1921; Green Weight of Roots and Shoots Produced by 1 Gram of Original Green Material*

Type of Cutting	Solution	Light or Darkness	Green Wt. of Roots in G. Produced by 1 G. of Original Green Material		Green Wt. of Shoots in G. Produced by 1 G. of Original Green Material	
			Basal	Terminal	Basal	Terminal
High carbohydrate.....	Plus nitrates...	Light....	0.2432	0.1631	0.3974	0.4041
		Darkness.	0.1357	0.0627	0.1590	0.0578
	Minus nitrates.	Light....	0.0116	0.0012	0.0001	0.0183
		Darkness.	0.0382	0.0097	0.0000	0.0020
Moderately vegetative....	Plus nitrates...	Light....	0.0013	0.0003	0.0617	0.1027
		Darkness.	0.0003	0.0000	0.0000	0.0064
	Minus nitrates.	Light....	0.0012	0.0024	0.0358	0.0467
		Darkness.	0.0011	0.0000	0.0121	0.0138

The most distinctive features of the growth responses may be summarized as follows:

1. Roots made their appearance on high-carbohydrate cuttings before there was any noticeable growth of shoots.
2. Shoots made their appearance on vegetative cuttings before there was any noticeable growth of roots.
3. Top cuttings of both vegetative and high-carbohydrate types produced a greater quantity of shoots than the basal ones when such cuttings were grown in the light.

TABLE 5. Series III, December 17, 1921, to January 10, 1922; Average Green Weight of Roots and Shoots Produced by the Individual Cutting of Average Weight

Type of Cutting	Solution	Light or Darkness	No. of Cuttings	Ave. Wt. of Cuttings			Ave. Wt. of Roots per Cutting			Ave. Wt. of Shoots per Cutting		
				Basal	Middle	Terminal	Basal	Middle	Terminal	Basal	Middle	Terminal
High carbohydrate.....	Plus nitrates.....	Light.....	6	8.123	4.853	2.097	1.948	0.795	0.172	4.448	2.435	0.784
	Minus nitrates...	Darkness...	6	6.788	4.312	2.278	0.970	0.644	0.219	2.107	1.039	0.140
Vegetative.....	Plus nitrates.....	Light.....	6	8.997	4.716	2.164	1.376	0.299	0.124	0.467	0.711	0.627
		Darkness...	6	6.433	4.265	2.411	0.582	0.388	0.171	0.000	0.000	0.018
	Minus nitrates...	Light.....	2	9.527	12.108	7.399	0.004	0.000	0.000	0.074	0.014	0.874
		Darkness...	2	7.791	12.520	7.666	0.000	0.000	0.000	0.000	0.000	0.000
	Light.....	Light.....	2	9.569	16.007	9.202	0.000	0.000	0.000	0.135	0.343	1.062
		Darkness...	2	8.016	14.955	9.278	0.000	0.000	0.000	0.000	0.000	0.000

4. The basal cuttings of both vegetative and high-carbohydrate sorts produced more roots than the top cuttings when grown either in light or in darkness and in either kind of solution. Vegetative cuttings grown in the light in the solution lacking nitrates were an exception in that the top segments produced more roots than the basal ones.

5. Light was more favorable than darkness to the growth of shoots, and in most instances also to the growth of roots.

6. Organic nitrogenous materials used in the growth of both roots and shoots must have been synthesized from nitrate nitrogen in darkness as well as in light, since greater growth of roots and shoots occurred from cuttings grown in the solution containing nitrates than from those grown in the solutions lacking them.

### Series III: December 17, 1921, to January 10, 1922

Inasmuch as differences in the growth of roots and shoots from the basal and top cuttings of series I and II had developed, it was thought desirable to study these phenomena by the use of more exact methods of experimentation. Although in the previous experiments the longer stems had been cut into three segments, the middle cuttings were grouped with the top cuttings and no separate measurements of their growth were taken. In series III, records of growth were obtained separately for basal, middle, and top segments. Moreover, each cutting was numbered and grown by itself in a 250-cc. test tube one inch in diameter. The temperature at which the cuttings were grown varied from 18° to 20° C. As in series II, two lots of experiments were conducted, one in the light and the other in darkness.

TABLE 6. *Series III, December 17, 1921, to January 10, 1922; Green Weights of Roots and Shoots Produced per Gram of Original Green Material*

Type of Cuttings	Solution	Light or Darkness	Green Wt. of Roots Produced per G. of Original Green Material			Green Wt. of Shoots Produced per G. of Original Green Material		
			Basal	Middle	Terminal	Basal	Middle	Terminal
High carbohydrate....	Plus nitrates.	Light..	0.2398	0.1638	0.0823	0.5472	0.5017	0.3741
		Darkness..	0.1429	0.1492	0.0968	0.3105	0.2409	0.0616
	Minus nitrates....	Light..	0.1529	0.0633	0.0575	0.0519	0.1507	0.2897
		Darkness..	0.0906	0.0793	0.0707	0.0000	0.0000	0.0077
Vegetative...	Plus nitrates.	Light..	0.0004	0.0000	0.0000	0.0078	0.0011	0.1181
		Darkness..	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Minus nitrates....	Light..	0.0004	0.0000	0.0000	0.0141	0.0214	0.1154
		Darkness..	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

In order to obtain some idea as to the relative extent of the utilization of food reserves contained in the different types of cuttings grown under the various conditions, microchemical tests for starch and nitrates were made before and after the regenerative period.

The high-carbohydrate type of plants used for these experiments had stems 18 inches long and with little or no external evidence of axillary shoots. The high-nitrogen plants had the appearance of being more vegetative than similar plants used in series I and II. Microchemical tests also indicated that the plants contained less starch.

TABLE 7. *Series III, December 17, 1921, to January 10, 1922; Dry Weights of Roots and Shoots Produced by 1 Gram of Original Dry Material*

Type of Cutting	Solution	Light or Darkness	Dry Wt. of Roots Produced by 1 G. of Original Dry Material			Dry Wt. of Shoots Produced by 1 G. of Original Dry Material		
			Basal	Middle	Terminal	Basal	Middle	Terminal
High carbohydrate....	Plus nitrates.	Light..	0.0748	0.0544	0.0358	0.2392	0.2313	0.2247
		Darkness..	0.0741	0.0740	0.0832	0.1871	0.1696	0.0764
	Minus nitrates....	Light*.	0.0647	0.0323	0.0251	0.0245	0.0846	0.1508
		Darkness..	0.0407	0.0316	0.0256	0.0000	0.0000	0.0106

\* These cuttings were put into the solution containing nitrates four days prior to the termination of the experiment, and the shoot growth, particularly that of the basal cuttings, was almost wholly developed during this interval.

The behavior of the basal cuttings of this series has been described and illustrated by figures in a previous article (Reid, 17). Tables 5, 6, and 7 give the quantitative data for this series. The following statements briefly summarize the results.

#### *High-carbohydrate Cuttings*

Grown in the solution containing nitrates: (1) Basal cuttings rooted sooner than the top cuttings. (2) Top cuttings produced shoots slightly earlier than the basal cuttings. (3) Basal cuttings when grown either in light or in darkness produced a greater weight of roots and shoots per gram of original green material than the middle or top cuttings.

Grown in solution lacking nitrates: Basal cuttings when grown either in light or in darkness produced a greater weight of roots than the top or middle cuttings, but the top cuttings produced a greater weight of shoots than the basal or middle cuttings.

#### *Vegetative Cuttings*

Grown in solutions plus or minus nitrates:

In light: (1) Shoots grew rapidly in the early portion of the growing period but no roots developed. (2) Later in the growing period roots appeared, but considerably sooner on cuttings grown in the solution lacking nitrates than on those grown in the solution containing them. (3) By the end of the growing period the total weight of roots produced per gram of original green material by basal cuttings grown in the two solutions was approximately the same. The cuttings from the middle portion of the stem produced the most roots when grown in the solution lacking nitrates. These cuttings produced a greater weight of roots per gram of original green material than the basal cuttings grown under similar conditions. (4) Shoots produced by cuttings grown in the two solutions were almost equal in quantity but somewhat different in character.

In darkness, neither roots nor shoots were produced.

**Series IV: High-carbohydrate Cuttings February 7 to 27, 1922;  
High-nitrogen Cuttings February 9 to 17, 1922**

The cuttings were grown in 250-cc. test tubes with one cutting per tube as in series III. Different lots of cuttings were grown in light and in darkness at temperatures which varied from 19° to 21° C. The plants from which the high-carbohydrate cuttings were obtained were very small and had been slow-growing plants previous to being transplanted into the sand. The slow growth was supposed to be due to the short, cloudy days of November and December. The plants averaged from 5 to 7 inches in height and were correspondingly small in diameter. These plants were not cut into segments, but were defoliated and the apical bud was removed. No positive tests for nitrates in the plants were obtained at the time the cuttings were made. There was considerable starch in the basal portions of the stems but not nearly so much per cell as there had been in the previous lots of high-carbohydrate plants. The central portion of the pith of many of these plants contained very little starch. The quantitative measurements of the growth-responses are indicated in tables 8 and 9 and may be summarized as follows:

TABLE 8. *Series IV, February 7-27, 1922; Average Green Weight of Roots and Shoots Produced by the Individual Cutting of Average Weight*

Type of Cutting	Solution	Light or Darkness	No. of Cuttings	Ave. Wt. of Cuttings		Ave. Wt. of Roots per Cutting		Ave. Wt. of Shoots per Cutting	
				Entire Stems Used					
High carbo- hydrate (Feb. 7- 27).....	Plus nitrates.	Light...	6	2.226		0.276		0.924	
		Dark- ness...	6	2.272		0.202		0.027	
	Minus nitrates....	Light...	6	2.987		0.414		0.121	
		Dark- ness...	6	2.486		0.312		0.008	
				Basal	Terminal	Basal	Ter- minal	Basal	Ter- minal
Vegetative (Feb. 9- 17).....	Plus nitrates.	Light...	4	11.704	11.755	0.000	0.006	0.315	1.413
		Dark- ness...	4	11.965	11.289	0.000	0.000	0.000	0.000
	Minus nitrates....	Light...	4	8.656	9.083	0.035	0.074	0.451	1.292
		Dark- ness...	4	10.588	10.845	0.000	0.000	0.000	0.000

TABLE 9. *Series IV, February 7-27, 1922; Green Weights of Roots and Shoots Produced per Gram of Original Green Material*

Type of Cutting	Solution	Light or Darkness	Green Wt. of Roots in G. Produced by 1 G. of Original Green Material		Green Wt. of Shoots in G. Produced by 1 G. of Original Green Material	
			Entire Stems Used			
High carbohydrate (Feb. 7-27).....	Plus nitrates...	Light....	0.1241		0.4150	
		Darkness.	0.0891		0.0121	
	Minus nitrates.	Light....	0.1386		0.0405	
		Darkness.	0.1254		0.0032	
			Basal	Terminal	Basal	Terminal
Vegetative (Feb. 9-17).	Plus nitrates...	Light....	0.0000	0.0005	0.0269	0.1202
		Darkness.	0.0000	0.0000	0.0000	0.0000
	Minus nitrates.	Light....	0.0040	0.0081	0.0521	0.1422
		Darkness.	0.0000	0.0000	0.0000	0.0000

*High-carbohydrate Cuttings*

Grown in the solution plus nitrates:

In light, the ratio of weights of shoots to roots (2.92) is nearly comparable to that of total shoots to total roots of the basal plus middle plus top

In darkness, the cuttings became withered at the tips. The capacity for shoot-production in darkness was very much less than in cuttings of any of the previous series grown under similar conditions. It had, however, been noted previously that top cuttings produced shoots less abundantly than basal cuttings when grown in darkness in the solution containing nitrates.

Grown in the solution lacking nitrates:

In light, there was abundant root growth and all but one of the cuttings grew at least one shoot. Traces of nitrates were found in the cuttings at the end of the regenerative period.

In darkness, only one of the cuttings developed a shoot. Roots grew well.

#### *Vegetative Cuttings*

Grown in solution containing nitrates:

In light, some growth of shoots occurred, but those of the basal cuttings remained small. No roots were produced by the basal cuttings, and in the case of top cuttings no roots appeared until there was considerable shoot- and leaf-development.

In darkness, there was no growth of roots or shoots.

Grown in solution lacking nitrates:

In light, the behavior was similar to that of cuttings grown in the solution plus nitrates, except that roots were produced earlier and more abundantly in the solution lacking nitrates. The top cuttings produced twice as much root substance per gram of original green material as the basal cuttings. This response was correlated with a difference in carbohydrate content at the end of the growing period, the top cuttings having considerably more starch and sugar.

In darkness, neither roots nor shoots were produced.

#### Series V: May 4 to June 10, 1922

Tests were conducted in light and in darkness with both vegetative and high-carbohydrate cuttings. The cuttings were grown in 250-cc. test tubes. About one third of an inch was removed from the apical end of each top cutting.

The high-carbohydrate cuttings supposedly had a higher internal nitrogen content than had similar cuttings of previous series, since the plants from which the cuttings were obtained were allowed to grow for a longer time before being transferred to the sand. When the plants were about 8 inches high, they were transplanted from the small pots in which they had been growing into a plot of nitrogen-rich soil where they grew very rapidly for 18 days. During this time they became considerably taller, the stems became much larger in diameter, and many of the axillary buds grew into small shoots. These axillary buds were more fully developed toward the basal region of the stem than toward the top. After growing



in the rich soil for 18 days, the roots of the plants were thoroughly washed to remove traces of organic matter that might contain nitrogen and the plants were then planted in quartz sand in 10-inch pots, 2 plants per pot. Although these plants were exceedingly vegetative at the time of transplanting, it took them no longer to become high in carbohydrates than it took the less vegetative plants used in previous series, similarly treated. This may have been partially due to the larger size of the leaves of the more vegetative plants. At the end of one month, a greater amount of starch per cell had accumulated than had been found in any material used in previous tests. At the time of taking the cuttings, there were traces of nitrates in only 3 of the 16 top cuttings used, whereas 9 of the 16 basal cuttings made from the same plants contained traces of nitrates. The cuttings were grown in temperatures which fluctuated from 19° to 23° C.

TABLE 10. *Series V, May 4-June 2, 1922; Average Green Weight of Roots and Shoots Produced by the Individual Cutting of Average Weight*

Type of Cutting	Solution	Light or Darkness	No. of Cuttings	Ave. Wt. of Cuttings		Ave. Wt. of Roots per Cutting		Ave. Wt. of Shoots per Cutting	
				Basal	Terminal	Basal	Terminal	Basal	Terminal
High carbohydrate...	Plus nitrates.	Light....	10	9.232	3.246	3.599	1.296	12.871	6.860
		Darkness...	10	11.266	3.143	1.807	0.095	2.897	0.178
	Minus nitrates....	Light....	10	10.732	2.998	2.148	0.245	1.121	1.255
		Darkness...	10	9.357	3.020	2.116	0.115	0.361	0.069
Vegetative..	Plus nitrates.	Light....	4	13.077		1.438		7.824	
		Darkness...	4	12.444		0.000		0.000	
	Minus nitrates....	Light....	4	12.827		0.670		6.335	
		Darkness...	4	9.483		0.000		0.000	

One of the particular objects of this experiment was to study as accurately as possible the behavior of cuttings as individuals. In order to accomplish this end, it was necessary to grow a smaller number of cuttings. Small segments of the stem, obtained from different levels and from each plant used were numbered and preserved in 95% alcohol. Tests for nitrates were also made and recorded for each individual. At the end of the regenerative period, thin sections made from the material preserved in alcohol were used in testing for starch. These results were checked against those obtained by a study of sections of cuttings from the same plants selected from corresponding levels of the stem and obtained at the end of the growing period. Tests for nitrates were also made on sections

from the cuttings at the end of the regenerative period. The results were compared with those obtained on the same cuttings at the time the cuttings were made. In this way it was possible to estimate the relative loss of starch and the presence or absence of nitrates in individual cuttings grown under different conditions.

The quantitative results of the growth responses have been recorded in tables 11 and 12 and may be summarized as follows:

TABLE 11. *Series V, May 4-June 2, 1922; Green Weights of Roots and Shoots in Grams Produced by 1 Gram of Original Green Material*

Type of Cutting	Solution	Light or Darkness	Green Wt. in G. of Roots Produced by 1 G. of Original Green Material		Green Wt. in G. of Shoots Produced by 1 G. of Original Green Material	
			Basal	Terminal	Basal	Terminal
High carbohydrate.....	Plus nitrates...	Light.....	0.3899	0.3991	1.3942	2.1139
		Darkness...	0.1714	0.0303	0.2571	0.0566
	Minus nitrates.	Light.....	0.2001	0.0817	0.1045	0.4186
		Darkness...	0.2261	0.0381	0.0385	0.0229
Vegetative.....	Plus nitrates...	Light.....	—	0.1100	—	0.5983
		Darkness...	—	0.0000	—	0.0000
	Minus nitrates.	Light.....	—	0.0522	—	0.4939
		Darkness...	—	0.0000	—	0.0000

TABLE 12. *Series V, April 27-May 20, 1921; Dry Weight of Roots and Shoots Produced by 1 Gram of Original Dry Material*

Type of Cutting	Solution	Light or Darkness	Dry Wt. of Roots in G. Produced by 1 G. of Original Dry Material		Dry Wt. of Shoots in G. Produced by 1 G. of Original Dry Material	
			Basal	Terminal	Basal	Terminal
High carbohydrate.....	Plus nitrates...	Light.....	0.1985	0.2599	0.9944	1.4821
		Darkness...	0.0489	0.0144	0.0982	0.0269
	Minus nitrates.	Light.....	0.0632	0.0333	0.0560	0.2591
		Darkness...	0.0684	0.0126	0.0050	0.0132
Vegetative.....	Plus nitrates...	Light.....	—	0.2162	—	1.3342
		Darkness...	—	0.0000	—	0.0000
	Minus nitrates.	Light.....	—	0.0682	—	0.8810
		Darkness...	—	0.0000	—	0.0000

### *High-carbohydrate Cuttings*

Grown in the solution containing nitrates:

In light, some of the young shoots attained a length of 12 inches and the indications were that the cuttings could have grown continuously in the nutrient solution, perhaps to the flowering stage. Roots appeared at the bases of the regenerated shoots, far above the level of the nutrient solution. Most of the original starch reserves were removed from the cuttings, although small traces of starch were left in the xylem and outer pith cells. Nitrates were present in abundance in the young shoots.

In darkness, numerous roots and shoots developed. The leaf blades did not grow well in darkness, and did not long remain in a turgid condition after they stopped growing. New leaves were developed at the tips at the time the older ones at the bases of the young shoots were withering. Nitrates were not found in the young shoots or in the cuttings themselves, above the level of the nutrient solution. Considerable starch had disappeared, but not so much as from basal cuttings grown in the *light* in the solution plus nitrates. The total growth was very much less than in the light.

Grown in the solution lacking nitrates:

In light, numerous large, white, succulent roots developed. One or two small shoots grew from each cutting. It may be recalled that basal cuttings of series III grown under similar conditions produced no shoots until nitrates were added to the nutrient solution. It is suggested that the appearance of shoots on the cuttings of series V may have been because of the more vegetative condition of the plants at the time the cuttings were made. At the end of the growing period the cuttings contained no free nitrates above the level of the nutrient solution, and only traces below in the region of greatest root-development. The starch disappeared to a remarkable extent, considering the large amount that was originally present. However, not nearly so much was utilized in the basal cuttings as in the case of those grown in the solution containing nitrates.

In darkness, these cuttings developed a distinctly greater weight of roots than did similar ones grown in darkness in the solution containing nitrates. Half of the cuttings produced shoots of appreciable size. At the end of the regenerative period, traces of nitrates were found in the cuttings below the level of the nutrient solution, but none above. Much of the original starch content had disappeared, and the greatest utilization of stored starch was found in those cuttings which originally contained traces of nitrates.

#### *Vegetative Cuttings*

No tests were conducted with basal cuttings of the vegetative plants used in this series. The growth of the top cuttings appeared to differ somewhat from that of similar cuttings used in previous series, and the difference was supposed to be due, at least in part, to a lowering of the nitrate content of the nutrient solution.

Grown in solution containing nitrates:

In light, these cuttings produced an abundant development of shoots with large leaves which were soft in texture and of a uniform dark green color. No roots developed until after the cuttings had been in the nutrient solution for 7-10 days, but at the end of the growing period there was a large mass of roots which were long and branched. It was supposed that the new growth was largely made at the expense of carbohydrate materials synthesized by the leaves.

In darkness, there was no growth of shoots or roots.

Grown in solution lacking nitrates:

In light, although these cuttings produced roots sooner than similar cuttings grown in light in the solution plus nitrates, yet the total quantity of roots at the end of the growing period was less. The topmost buds and in some cases others near them developed large-sized shoots with pale yellowish-green leaves, relatively stiff in texture.

In darkness, neither roots nor shoots developed.

#### Series VI: April 2 to 16, 1923

#### Series VII: April 15 to May 8, 1923

Two series of experiments were conducted with vegetative cuttings to study more fully their behavior under varying external conditions than had been possible in previous series because of the limited number of vegetative plants that had been available for any one experiment. The experimental conditions were the same as were employed for series V. Eight cuttings were used for each individual test. Some of the chemical changes with particular reference to the carbohydrates were followed. Weights of cuttings and of regenerated structures were not obtained, as the material had to be freely used for microchemical tests. The plants from which the cuttings were secured were exceedingly vegetative. Branches had developed from the nodes of the basal portions of most of the stems. Microchemical tests conducted on some of the stems showed very little reserve starch in the basal region, but considerably more in the middle unbranched region and slightly more in the terminal region.

The top cuttings grown in the light produced more shoots than did the middle cuttings or the unbranched basal cuttings when grown in solutions either containing or lacking nitrates. The much-branched basal cuttings were considered more like top cuttings in composition because of their large amount of young shoot-growth. The branched basal cuttings behaved much like the top cuttings and produced shoots abundantly when grown in either of the solutions.

There was some variation in the amount of roots produced by the different segments. In most instances, roots developed in greatest abundance from the middle cuttings in which considerable starch and sugar were present. There were indications that the place of cutting, whether above or below a node, was of importance for the middle and top vegetative cuttings, particularly the latter. They rooted slightly more quickly when the cutting was made with the basal end just above a node rather than just below one. This was interesting partly because of the fact that a higher sugar content had been found in highly vegetative tomato plants just above than just below the leaves. The tests were made after the plants had been kept in bright sunlight for several hours.

In the case of vegetative cuttings grown in darkness, it was usually the middle segments that developed a greater amount of shoots than the much-branched basal segments. The latter behaved like the top segments in that they did not grow and after a few days began to wither at the tips of the branches. It seemed that the greater the amount of young, actively growing tips present in the original vegetative cuttings the more favorable were the chances for shoot-growth in the light, and the smaller the amount of such growing tips in the original cutting the more favorable were the chances for shoot-growth in darkness. A few tests conducted in darkness with entire defoliated stems have indicated that the withering of the tips of the branches did not occur so readily as it did when young portions were detached from the rest of the stem. If growth occurred in these entire stems, it was usually from the apical buds or from others near them rather than from buds located on the more mature portions of the stems. It seemed that the apical buds inhibited the development of the buds in the older, less active regions if the stem was entire, but that the apical buds themselves could not grow in darkness if they were detached from the apparently more mature regions.

#### Series I-VII

##### *Character and Distribution of Shoots and Roots under Different Environmental Conditions*

Shoots produced by cuttings high in carbohydrates, grown in the light in the solution plus nitrates, were stocky and had well developed xylem with marked thickening of the cell walls. The leaves were large and medium-dark green in color. Shoots produced by similar cuttings grown also in the light but in the solution lacking nitrates were stiff, crisp, and yellowish-green in color.

The vegetative cuttings grown in the light in the solution plus nitrates produced large, succulent shoots, thickly covered with glandular hairs and with a relatively large amount of pith and with less xylem than the high-carbohydrate cuttings grown under similar conditions. The cell walls of the former were thinner. The leaf blades were very soft and intensely green in color. Their edges were much curled under as though the upper surface had grown faster than the under surface, and the mesophyll externally had a cushiony appearance which suggested that its cells had grown at a more rapid rate than had those of the contiguous veins. The cells of the palisade tissue were small, densely crowded, and somewhat larger in diameter at the surface adjacent to the upper epidermis than at their opposite ends. They were densely filled with protoplasmic contents.

The vegetative cuttings grown in the light but in the solution minus nitrates produced much stiffer shoots, which were also very hairy. The leaves were moderately bright green and at first soft, but later became more yellowish and stiffer. The edges did not curl under and there was

no puffiness of the mesophyll. The veins were much more prominent than in those just described. The cells of the palisade tissue were larger and not so densely filled with protoplasmic contents.

There appeared to be differences in both external and internal characters of shoots produced by vegetative and high-carbohydrate cuttings grown in darkness, but it seems desirable to conduct more detailed studies upon the differentiating characters before attempting to describe them.

The roots produced by high-carbohydrate basal cuttings grown in solutions either plus or minus nitrates were very numerous, but usually rather short and not profusely branched. The latter characteristic held particularly for roots developing from cuttings grown in the solution lacking nitrates. The roots of middle cuttings of the same plants were fewer, much longer, and more branched. The roots of the top cuttings were still fewer and appeared somewhat later, but were also long and profusely branched. The roots of all the high-carbohydrate cuttings, in general, were rather stiff, with considerable thickening of the cell walls particularly of the vascular tissue.

Extremely vegetative cuttings produced roots which were short and not profusely branched. Under conditions of a somewhat higher carbohydrate supply, the roots produced by vegetative cuttings were longer, larger in diameter, slightly more numerous, and considerably branched. With a still higher supply of carbohydrates and an abundance of nitrogen the roots were very long, profusely branched, and the cell walls, particularly those of the vascular tissue, were somewhat thicker.

The distribution of the major portion of the roots and shoots was to a large extent conditioned by the level of the nutrient solution on the cuttings. However, there was frequently a notable tendency for roots and shoots to develop at the same level on the stem. High-carbohydrate basal cuttings grown in the solution plus nitrates frequently had roots distributed from the lower nearly to the upper ends even above the level of the nutrient solution. Shoots grew from all the nodes, even from those below the level of the nutrient solution and in the region of greatest root growth. The tendency toward a production of roots and shoots at the same level on the cutting and throughout its length was particularly evident in the early part of the growing period. Later, a more definite polarity tended to become established, resulting in the production of shoots above the level of the nutrient solution and of roots below it.

The roots were produced at both nodes and internodes. Although they were more numerous along the lines of the primary vascular bundles, they were, nevertheless, generally distributed. On the middle and top high-carbohydrate and vegetative cuttings, in which the root "protuberances" were usually not noticeably developed, the roots tended to be localized at the margin near the wound, near the bases of the cuttings in the region of the lowest node, and in lines upward corresponding to the primary vascular bundles.

## DISCUSSION

The foregoing experimental results have furnished new evidence in support of the concept that the character of the growth response of plants is a direct expression of a measurable qualitative and quantitative condition of food and nutrient materials within the plant.

In all the experiments and in any one lot of like cuttings, similarly treated, those individuals which had the greatest original weight produced the largest total growth of roots or shoots or both. The character of the response in growth is not merely a quantitative relation of the reserve food materials; it is also dependent upon the nature of these materials. In comparing the results of one experiment with those of another, neither the same total amount of growth nor the same relative amounts of roots and shoots are necessarily produced for each gram of the original cutting material, whether the weight is reckoned in terms of green or of dry weight. When the carbohydrate content of a cutting is very high in relation to its nitrogen content, an abundance of roots, but few, and in many cases no, shoots are produced. If the relationship of the materials is reversed, there is an early production of shoots; but roots do not develop until some time later, when the internal composition with respect to the relative proportion of carbohydrates to nitrogen has notably changed.

The character of growth in response to nutrient materials supplied the cutting depends on the kinds of materials within the cutting. If nitrates are absorbed by cuttings high in carbohydrates, there is an almost immediate response in growth, particularly noticeable with respect to growth of shoots. If nitrates are absorbed by a cutting already high in total nitrogen but low in carbohydrates, there is a marked difference in behavior depending on whether the cuttings are kept in light or in darkness. In the light these cuttings produce as much total growth without nitrates as with them during the first two weeks of growth. When nitrates are used, there is at the close of the growing period very little growth of roots, not much growth of stems, but an abundant growth of leaves, particularly of the mesophyll tissue. When grown without nitrates, more roots are produced and the amounts of leaves are often nearly the same, but the veins are much heavier and more prominent and the mesophyll tissues are less prominent.

In darkness the top vegetative cuttings become withered at the tips after a few days whether nitrates are supplied or not, although the effects are noticeable somewhat sooner when nitrates are supplied. Harvey and True (8) have reported harmful effects of magnesium nitrate in concentrations higher than  $125 \text{ } N \times 10^{-6}$  upon squash seedlings grown in darkness, and they attribute the detrimental effects to the magnesium ion. It is possible that a toxicity may arise also as a result of a high concentration of nitrate ions. It is suggested that certain products of metabolism may accumulate more abundantly in the presence of nitrates and a low concentration of soluble carbohydrates, and that these metabolic products become

in relatively high concentrations.

Suzuki (23) has found that, if there is an abundance of available sugar, potato shoots detached from the tubers and grown in darkness can use nitrates in the formation of asparagin, although there is no increase of albuminoid nitrogen and no growth. If there is very little sugar available, the nitrates are not utilized, although there is no injury. In that case urea is a better form of nitrogen. Urea need not be reduced as must nitrates by substances such as sugars, but can be built directly into substances such as amino acids. Mr. Aoyama (23), of Suzuki's laboratory, has conducted experiments "which show clearly the poisonous action of ammonium salts, when the necessary amount of sugar is not present to transform them into asparagine."

In the experiments here reported, the high-carbohydrate cuttings, particularly the basal ones, frequently show the primordia of numerous adventitious roots, so far grown out into the cortex that they appear as distinct elevations. The axillary buds, however, are in most cases small, and sometimes are not visible externally. The cuttings from vegetative plants, on the contrary, have buds more fully developed; in some cases large branches are present but the development of the young roots is very slight. It might be supposed that those structures which are the most advanced would have the greatest advantage in competing for foods and food materials. Although this may be true, there are many indications that any advantage is overcome during the regenerative period, supposedly by the changing balance and composition of the available foods. Some of the high-carbohydrate cuttings, such as those used in series V for example, which have young roots and buds, both rather fully developed, produce mainly roots when no nitrates are supplied. Certain of the basal vegetative cuttings, which have both prominent young roots and buds, regenerate shoots chiefly.

As another example, some young tomato plants grown at the Boyce Thompson Institute were allowed to remain in 5-inch pots containing a loam soil until they became pot-bound. The axillary shoots had developed considerably, some of them being an inch long, although few primordia of the adventitious roots were visible. The stems showed a large reserve of starch and much woody tissue. Basal cuttings made of these plants, grown without nitrates in diffuse light, produced mainly shoots during the first two weeks, but later the roots began to develop profusely. At the time the experiment was terminated, the total mass of roots per cutting was two to three times as great as that of the shoots.

Because of the small supply of reserve carbohydrates in vegetative cuttings, it is difficult with the methods used to follow quantitatively the changes in the carbohydrate supply during the successive stages of development. When cuttings contain a large carbohydrate reserve, however, there is generally a greater utilization of starch when the cuttings are supplied with nitrates and grown in the light than when no nitrates are



supplied or than when the cuttings are grown in the dark with or without nitrates.

Since there is growth of roots or shoots or both from most of the cuttings grown in the solution lacking nitrates, a utilization of certain reserve nitrogenous materials within the cuttings must occur. At the close of the growing period of some of the high-carbohydrate cuttings, the diphenylamine test gives a positive result for nitrates, although the material gives no such result at the time the cuttings are made. Toole (24) germinated corn grains in conductivity water and observed a distinct accumulation of nitrates in the mesocotyl just below the first node.

Marked differences have been observed in the behavior of cuttings from different levels of the stem with respect to the rate and relative total growth of roots and shoots. It would seem that the responses are in some way related to the carbohydrate and particularly to the nitrogen gradients in the stem. In general, the gradient in nitrogen increases from the base to the apex of the stem and the gradient in carbohydrates increases from the apex to the base. In these experiments, high-carbohydrate apical cuttings displayed a greater capacity for the production of shoots on the basis of their own internal nitrogen reserves than the basal ones. On the other hand, the basal high-carbohydrate cuttings show a greater capacity for root-production than the apical ones. In the high-carbohydrate cuttings gradients thus appear to exist in the capacity for shoot- and root-production when the cuttings are grown on their own nitrogen reserves.

Gradients in capacity for root- and shoot-production are also found for vegetative cuttings obtained from different levels of the stem. It is found that those cuttings that have the greatest carbohydrate content have the greatest ability to produce roots. The moderately vegetative plants of series I have gradients in carbohydrate content increasing from the apex to the base. The greatest ability to produce roots is displayed by the basal segments of these plants. The extremely vegetative plants of series IV, VI, and VII have gradients in carbohydrate content increasing from the apex and base toward the middle regions of the stem. The middle cuttings of these stems have the greatest ability to produce roots, and the top cuttings and much-branched basal cuttings have the greatest ability to produce shoots.

We thus see that there are gradients in the capacity for root- and shoot-production by both highly vegetative and high-carbohydrate cuttings, although the gradients are different in the two cases. Are we then to suppose that high-carbohydrate stems possess gradients in root-forming substances increasing from above downward, and gradients in shoot-forming substances increasing from below upward; and that highly vegetative stems have gradients in root-forming substances increasing from above and below toward the middle region of the stem and of shoot-forming substances increasing from the middle toward the top and toward the much-branched

basal region? My results seem to show that this is the case with the plants used in these experiments.

It must also be that separation of the formative materials is never quite complete, since roots and shoots grow from the same level of the cuttings and in close proximity. It may be recalled that this is one of Robertson's (21) chief objections to the conception of specific foods for shoots and roots.

There appears to be rather definite evidence that certain relatively simple materials whose exact nature is not known, although apparently related to such substances as sugar or other non-nitrogenous carbon compounds and inorganic nitrogen compounds, are used in the growth of both roots and shoots but in somewhat different proportions. Future work must concern itself with the more specific determination of some of these compounds and with attempts at discovering how differences in the development of the plant organs are initiated or continued.

#### SUMMARY

1. A high nitrogen supply plus a readily available supply of carbohydrates appears to furnish favorable conditions for growth of shoots.

2. A somewhat limited nitrogen supply plus a relatively large supply of readily available carbohydrates appears to furnish favorable conditions for the growth of roots.

3. The following are the characteristics of roots and shoots produced by cuttings when grown under conditions supplying varying quantities of available carbohydrates and nitrogen:

- A. High-carbohydrate and low-nitrogen supply: *Shoots* small, both as to number and size, rigid, bearing stiff, yellowish-green leaves. *Roots* very numerous, frequently unbranched, usually not long.

- B. High-carbohydrate supply and somewhat greater supply of nitrogen than in A: *Shoots* more numerous than in A, darker green leaves, somewhat softer in texture. *Roots* very numerous, usually branched, longer than in A.

- C. Carbohydrate supply somewhat limited but an abundance of nitrogen: *Shoots* numerous, large, succulent, with large leaves, soft in texture. *Roots* fewer than in A and B, but longer and more branched.

- D. Low-carbohydrate and high-nitrogen supply: *Shoots* succulent, thick, intensely green leaves, having the edges usually curled under and the mesophyll areas cushion-like. *Roots* very few, short, often unbranched.

4. A more complete utilization of the carbohydrate reserves occurs when high-carbohydrate cuttings are grown in the light than when they are grown in darkness. The utilization of carbohydrates appears to be more rapid in solutions containing nitrates than in those lacking nitrates.

5. When a supply of reserve carbohydrates is present, synthesis of nitrogenous materials that can be used in growth occurs in cuttings grown in the solution containing nitrates both in light and in darkness, but such synthesis appears to be greater in amount in light than in darkness. In

the case of cuttings kept in darkness, growth stops and death results at the end of several weeks, although a supply of carbohydrates is still available. These latter results indicate that light is necessary for the synthesis of certain materials, other than carbohydrates, used in relatively small amounts in the growth of roots and shoots.

TABLE 13. *Ability of Different Segments of the Stem to Produce Roots and Shoots when Grown in the Light; Letters B, M, and T Indicate which Segments Produced the Greatest Weight of Roots and Shoots (Basal, Middle, or Terminal); Data Based on Growth Produced per Gram of Original Green Material*

	Composition of Cuttings	Series	Roots		Shoots	
			With Nitrates	Without Nitrates	With Nitrates	Without Nitrates
High-carbohydrate cuttings..	Moderately high-carbohydrate, moderately low-nitrogen.....	I	B	B	B	T
	High-carbohydrate, low-nitrogen.....	II	B	B	T	T
	High-carbohydrate, low-nitrogen.....	III	B	B	B	T
	Very high-carbohydrate, moderately low-nitrogen.....	V	T	B	T	T
Vegetative cuttings.....	Moderately low-carbohydrate, moderately high-nitrogen.....	I	B	B	B	B
	Moderately low-carbohydrate, somewhat higher in nitrogen than I.....	II	B	T	T	T
	Lower in carbohydrates than I and II and higher in nitrogen.....	III	B	M	T	T
	Very low-carbohydrate, very high-nitrogen....	No middle cuttings				
		IV	T	T	T	T
	Very low-carbohydrate, very high-nitrogen....	VI	M	M	T	T
	Very low-carbohydrate, very high-nitrogen....	VII	M	M	T	T

6. Shoots are produced in greater amounts in light than in darkness. A greater quantity of roots is produced in darkness than in light by high-carbohydrate cuttings with a moderately high internal nitrogen reserve and grown in the solution lacking nitrates, but if high-carbohydrate cuttings with a lower nitrogen reserve are grown under the same conditions, a greater quantity of roots is produced in light than in darkness.

7. The greater the amount of very young tissue in cuttings, the greater is their ability to produce shoots when grown in the light.

8. The smaller the amount of very young tissue in cuttings, the more favorable are the conditions for shoot-production in darkness.

9. The ability of cuttings from different levels of the stem to produce roots and shoots varies with the composition of the cuttings and also with the external conditions under which they grow. The data are summarized in table 13.

10. The evidence at hand points to the nature and quantities of the available organic food materials as being of special significance in influencing the growth of roots and shoots.

These investigations, begun at the suggestion of Professor E. J. Kraus, were conducted in the Department of Botany at the University of Wisconsin. I wish to extend grateful acknowledgment to Professors J. B. Overton and E. J. Kraus for helpful suggestions and criticisms given during the progress of the work.

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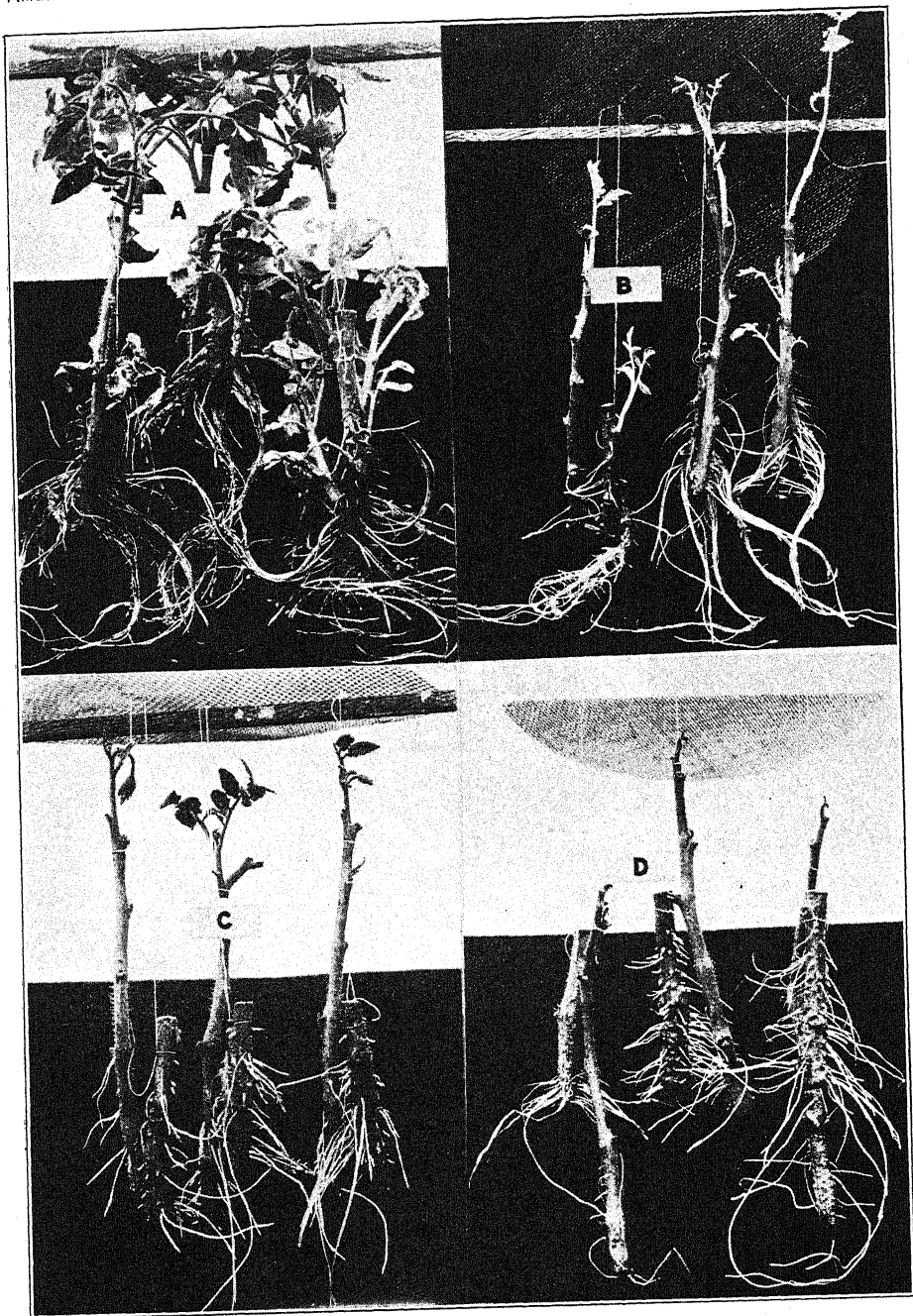
## DESCRIPTION OF PLATES

### PLATE XXXVIII

Basal and terminal cuttings of plants high in carbohydrates. Cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness. Cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.

### PLATE XXXIX

Basal (1), middle (2), and terminal (3) cuttings of vegetative plants. Cuttings grown in solution plus nitrates: *A*, in light; *B*, in darkness. Cuttings grown in solution minus nitrates: *C*, in light; *D*, in darkness.



REID: GROWTH OF TOMATO CUTTINGS





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## A BIOLOGIC AND PHYLOGENETIC STUDY OF THE STROMATIC SPHAERIALES<sup>1</sup>

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### INTRODUCTION

Taxonomy should be an expression of phylogeny. A knowledge of the phylogeny of any group of organisms, however, must lag behind the necessity of its classification. The mere existence of a number of described species calls for a taxonomic system. A knowledge of the phylogenetic relationships comes only after a close scrutiny of the structure and development of these species. For this reason the first systems of classification of any group are necessarily artificial.

This is the case with the ascomycetous fungi. The enormous number of described species has necessitated the erection of systems of classification which are based upon arbitrarily chosen characters, resulting in a high degree of artificiality. Lindau (79) has long since pointed out that the only way to bring order to this mass of unassimilated species is to desist from the wholesale formation of species and to proceed to a study of the life histories of the forms already known.

The problem here undertaken is the determination of the phylogenetic relationships existing among certain ascomycetes, and the presentation of the outlines of a possible classification upon such a basis. In undertaking such a problem it is necessary to have, first of all, a thorough knowledge of the complete life histories of a large and representative number of species. This is especially true of the ascomycetes on account of their complicated life histories, which include two or more modes of spore-formation. In order to make this possible, the study is here limited to a reasonably well-defined group, the stromatic Sphaeriales. Since the only dependable method of determining the genetic connections of the various spore forms belonging to a given life history is by pure-culture methods, the life histories of a number of closely related forms of the stromatic Sphaeriales have been worked out in culture by the writer (127, 128, 129, 130). In the following pages an attempt will be made to correlate the results of these

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investigations, together with what other data are available, into a unified whole as regards the general developmental morphology and phylogenetic relationships of this group.

This work has been done at the cryptogamic laboratory of the University of Michigan under the direction of Dr. C. H. Kauffman, to whom I wish to express my extreme gratitude for the suggestion of the problem and the continual helpful supervision of the work.

### HISTORICAL

The study of the fungi was much later in its development than that of the higher plants. The earlier writers had very vague ideas of the cryptogams, and generally supposed them to arise by spontaneous generation. As in the study of the higher plants, the work of the earlier mycologists, such as Persoon (87) and Fries (34), was mainly descriptive. The collectors of the earlier part of the nineteenth century described an enormous number of species, which were assembled in highly artificial systems of classification. A comprehensive knowledge of the fungi did not begin to develop until the middle of the nineteenth century, when the perfection of the microscope made minute microscopic studies possible and the researches of Hofmeister focused attention upon the lower cryptogams.

When the earlier systems of classification of the fungi were erected, the knowledge of the spore and its morphology was very slight, and as a result separations were based mainly on the gross morphological characters. In the Sphaeriales, the character and position of the perithecium and the configuration of the stroma became the chief diagnostic characters.

In 1851, a great stimulus was given to the study of the ascomycetes in general by the discovery, by L. R. Tulasne (120, 121, 122), of the "spermatogonia" and "spermatia" in both the lichens and the ascomycetes. His suggestion that these organs play some part in sexual reproduction, and might be the male reproductive organs, resulted in numerous investigations by de Bary (7), Cornu (21), Brefeld (13), and others of this question. These investigations have gradually discredited Tulasne's hypothesis, but it still persists in the back-ground of the biology of these fungi. A second important result of this part of Tulasne's work was the establishment of the pleomorphism of the ascomycetes, that is, the occurrence of two or more spore forms in the life history of one and the same fungus. In the three volumes of the "Selecta Fungorum Carpologia" of L. R. and C. Tulasne (123), are to be found excellent plates and numerous descriptions of their ideas of certain life-history connections of the conidial and perithecial stages of these fungi. Although based on observation and inference only, most of these life-history connections remain the most authentic we have for the species concerned.

In 1867, Füsting (36) published the first studies upon the developmental morphology of the stromatic Sphaeriales. He studied the develop-

ment of the stroma and perithecium in a number of species, and found coils of large, deeply staining hyphae, which he termed "Woronin hyphae," and which occurred in the perithecial initials of *Diatrype* (*Diatrypella*) *quercina*, *Eutypa*, and other forms.

In the latter half of the nineteenth century, there appeared a number of works on the taxonomy of the Pyrenomycetes; these systems were based on the accumulation of knowledge of the morphology and life histories of these forms since the time of Persoon and Fries. The systems of Nitschke (83), Fuckel (35), and Winter (132) in Europe, and of Ellis (29) in America, were based chiefly on the position, structure, and arrangement of the perithecium and stroma; these characters were used to distinguish the families of the Sphaeriales, while spore characters were generally used for generic separations. Saccardo (100), on the other hand, used spore characters for the separation of very large groups. These systems still remain as the principal classifications of the Sphaeriales.

In 1891, Brefeld (14, 15) published the results of extensive cultures of the stromatic Sphaeriales and of the ascomycetes in general. In 1900, Ruhland (99) undertook a study of the development of the stromatic forms of the Sphaeriales, and attempted to erect a phylogenetic scheme upon the basis of the development of the stroma.

From 1900 to the present time there have been numerous cultural studies of the life histories of various species of ascomycetes. Very few of these studies, however, have been of the stromatic forms. Most of the work has been upon more or less parasitic forms of economic importance. The work of Klebahn (67, 69, 72), Potebnia (95, 96, 97), Stone (113, 114, 115), Laibach (73, 74), Miles (81), and others on *Mycosphaerella*, *Gnomonia*, and other genera; of Miss Stoneman (116), Shear (107, 111), and Edgerton (26, 27) on *Glomerella*; of Harter (44, 45, 46) and Miss Jenkins (65) on *Diaporthe*; and of Wollenweber (134) on the perfect stage of *Fusarium* may be cited as examples of this type of work.

During this same period a second group of workers appeared in Europe, whose attention was focused on the morphology and taxonomy of fungi in general, and of the ascomycetes (including their imperfect stage) in particular. Chief among these were von Höhnelt, H. and P. Sydow, Theissen, Petrak, Rehm, and others. These workers were in touch with the large herbaria of Europe, and were able to study enormous numbers of specimens and to compare them with authentic type material of earlier workers. As a result of their work there is available a vast amount of technical data concerning the synonymy and detailed morphology of these fungi. The important result of the sifting of these data has been the recognition of certain broad relationships within the Pyrenomycetes.

The work of these men brings us to the present time. The systems of the earlier workers, since they were based upon a comparatively incomplete knowledge of the forms in question, was necessarily artificial and temporary.

The great mass of work on the Sphaeriales since that time has been in the shape of scattered and uncorrelated descriptions of species and genera. There has arisen, however, a large amount of information on both the cultural and the morphological sides, and it seems to the writer that the time is ripe for a new attempt to recast our knowledge of these forms along the lines of their natural relationships.

#### MORPHOLOGY AND TERMINOLOGY

Before entering upon a discussion of phylogeny it is necessary to understand the organisms that one is to consider. One needs at the outset, therefore, a clear idea of the structural morphology, biological characteristics, and terminology of the groups involved. Perhaps the most important point to be understood, before entering upon a discussion of the stromatic Sphaeriales, or of the fungi in general for that matter, is the extreme plasticity of their morphological characters. In these stromatic forms, the fruit bodies are surrounded by more or less differentiated vegetative hyphae. Slight variations in the character of the substratum, or in the factors affecting growth, cause variations in the type or amount of this mycelium produced. Such differences in growth often result in wide variations in the morphological characters of any species. In contrast to this rather wide individual variation, we find that the range of variation of the entire group is comparatively restricted in respect to just those characters which are widely variable in the species. These facts, together with that of the large number of species, mean that there are numerous intergrading and overlapping forms. Such a situation necessitates the consideration of very minute differences. There has been an all-too-great tendency, however, to separate species and genera without a sufficient conception of what the variation within a given species or genus might be under different conditions. Probably the best morphological example of this variation within a species is that of the stroma. It is the stroma which is supposed to be characteristic of the group here to be considered; and its variable structure is responsible for the numerous transitional forms occurring between so-called species.

Before going further, let us determine what the term *stroma* signifies. The term has had a widely varying usage. An examination of Orton's paper (86), which gives the history of this usage, will show the broad application of the term by various writers in the past. In examining this usage we find that the term *stroma* has been used most generally in the ascomycetes, and has been applied generally to the vegetative matrix of a fruiting structure. Such matrices are merely aggregations of vegetative mycelium and differ from the ordinary mycelium only in the compacting or coalescence of the hyphae. Sclerotia, and other sterile masses or bodies, *not associated with spore-bearing structures*, are identical in their histological nature with the matrices of various compound fruit bodies, and should be included in any definition of a stroma. Finally, as pointed out by Orton,

the tissues arising as a result of a sexual stimulus should be excluded from the conception of this term.

Ruhland (99) defines *stroma* (as distinguished from *mycelium*) as "die Gesamtheit derjenigen vegetativen [in contrast to the fruiting mycelium of the perithecium and the conidial hymenium] Bestandtheile des Pilzkörpers, welche ohne ausschliesslich der Resorption zu dienen, sich in irgend welcher Weise am Aufbau des Fruchtkörpers betheiligen." This definition, however, limits the term *stroma* to components of a compound fruiting body (see definition, p. 17), and does not sharply distinguish those tissues of a sexual fruit body which arise as a result of a sexual stimulus.

With these points in mind, let us define a stroma as an aggregation of vegetative mycelium not resulting from a sexual stimulus.

This definition is admittedly very broad. Numerous other terms have arisen, however, to designate the various types of stromatic structures and aggregations of mycelium, and it is considered that the term *stroma* should be used in this broad sense, to distinguish a type of mycelial differentiation rather than any particular morphological structure.<sup>2</sup> Such an aggregation of mycelium may be merely a loosely compacted mass; it may be a mixture of fungous hyphae and substratum tissue, or host tissue; or it may be a solid mass of fungous pseudoparenchyma or pseudoprosenchyma. Such a tissue may be symphogenous or meristogenous in origin. It may, or may not, have an outer differentiated crust. The type of cells, the darkened color of the cell walls, or its morphological structure does not alter its stromatic nature.

This definition, however, excludes tissues arising as the result of a sexual stimulus, such as the wall of the perithecium or the hypothecium of an apothecium. It also excludes such reproductive tissue as hymenial layers or other sporulating tissues, and does not include the purely nutritive mycelium of the vegetative portion of the plant.

Both Füsting (36) and Ruhland (99) have recognized the differentiation of the stroma of many twig-inhabiting fungi into two tissues, which they have designated as *epistroma* and *hypostroma*, and *ectostroma* and *entostroma*, respectively. The application of these terms by both writers clearly indicates differences in position and structure. These writers themselves, as well as others, have confused these terms, however, with *conidio-stroma* and *ascostroma*, and have attempted to attribute to them a difference in function in their relation to spore-production. It is true that conidia are usually produced from ectostromatic tissue, and that perithecia ordinarily arise in the entostroma. In the majority of perithecial stromata, however, the ectostroma remains sterile and serves merely the mechanical function of rupturing the periderm. Furthermore, in many cases, as in

<sup>2</sup> This definition is applicable chiefly to the ascomycetes. The term is of such restricted usage in the basidiomycetes and other fungi that no attempt will be made here to discuss its meaning in those groups.

Eutypella or the subgenus Leucostoma, the conidial locules ordinarily arise within the entostromatic tissue. These terms will be limited, therefore, to designate differences in structure and position, and not in function. They may be defined as follows:

An *ectostroma*, in the Pyrenomycetes, is that portion of the stroma which is formed on the surface of the bark, beneath or within the periderm, and which consists typically of fungous tissue only, except that when it is developed within the periderm it may contain the remnants of the periderm cells, but never of the bark cortex cells.

An *entostroma*, in the Pyrenomycetes, is that portion of the stroma which develops within the cortical or woody tissue of the host or substratum and is made up of components of both fungous and host tissue or substratum tissue.

These terms were coined for tissues produced in a group with a special type of development, and can not be applied to all fungi, any more than the term *perithecium* can be applied to the Discomycetes.

The ectostromatic and entostromatic tissues, in some species, as in *Diatrype stigma*, are sharply differentiated. It must be remembered, however, that the ectostroma arises from the same mycelium within the bark tissues which proliferates to form the entostroma, and therefore in many cases, as in certain species of *Diaporthe*, the distinction between these two tissues is not sharply defined. In other cases, as in the genus *Valsaria*, the entostromatic mycelium becomes so strongly developed that it forms an erumpent stroma composed largely of fungous tissue, which may superficially resemble an ectostromatic structure. Wherever we attempt to apply sharply defined terms to living organisms this same situation will be met. There will always be variations which can not be rigidly interpreted. Neither does a multiplication of terms help this situation. The use of qualifying statements is necessary for these intergrading forms.

Let us now turn to the origin and development of the stroma in the Sphaeriales, in order to trace its variation in the different groups. The stromatic Sphaeriales have apparently arisen from the simpler forms of the Pyrenomycetes by acquiring the habit of forming their perithecia immersed in the host or substratum tissue. This habit, in turn, could not be acquired until the mycelium had become capable of penetrating this tissue. Just as all fungous structures are made up of mycelial elements, so, likewise, has the stroma arisen through a development and differentiation of the immersed mycelium. The first evidence of a primitive stroma appears as a blackening of the surface of the substratum. Such a condition may be seen in many species of *Eutypa* and of the subgenus *Euporthe*. The next step in stromatic development comes about by the proliferation of the mycelium within the substratum. As this formation of mycelium increases, it usually becomes more or less localized about the forming perithecia. With this differentiation of an entostroma, there is usually correlated, very

often beneath a differentiated ectostroma, a clustering of the perithecia. This mode of organization of a compound fruiting structure is in reality the formation of a stroma immersed within the bark tissue. Just as most superficial stromata form an outer layer of darkened thick-walled cells upon contact with the air, so does this immersed stroma form a zone of similar blackened tissue about its margin. This is the blackened zone outlining the stromatic areas, which is so common in the stromatic Sphaeriales. This marginal zone of blackened tissue protects the stromatic areas from the absorptive action of the nutritive hyphae in the adjoining bark tissues, as has been shown by the writer for *Cryptosphaeria populina* (Pers.) Sacc. (129). The entostromatic hyphae themselves, which bind together the bark cells of this area, act as a protective and nutritive tissue for the developing perithecia.

As one passes from the most primitive to the most highly developed stromatic forms, these various stromatic characters show all degrees of variation in the different species. These variations in stromatic configuration have been largely used as characters for the separation of various groups. Ruhland (99) attempted to work out the phylogeny of this group on the basis of the stromatic development. His reasoning was based chiefly on the type of tissue concerned in, and the manner of, the bursting of the periderm. These are valuable diagnostic characters, but, in the writer's opinion, Ruhland's attempt has failed for two fundamental reasons. In the first place the phylogeny of no group can be determined from one set of characters alone, without the consideration of the development and correlation of other characters. Secondly, the stroma of the Sphaeriales has arisen and developed independently in several separate groups, in a more or less nearly parallel manner.

A number of terms have been used to describe the various types of stroma. Any particular stromatic configuration, however, is made up of a number of characters which vary independently. As a result we may have any combination of, or variation between, these characters. In a discussion of relationships it is more clear, therefore, to speak of the stroma in terms of these varying characters than to speak of fixed types of stromata (as Valsoid or Eutypoid) which designate combinations of such characters fitting only special cases. It is hoped, therefore, that the following account of the terms to be used for these characters will aid in a clear understanding of what follows.

The term *compound fruit body* will be here used to designate the type of fruiting structure which consists of an aggregation of a number of perithecia seated upon, included within, or oriented beneath, a stromatic tissue. In its highest development, in the forms here discussed, such a compound fruit body consists of a number of perithecia included within a differentiated entostromatic tissue and oriented beneath an erumpent ectostroma. This type of fruit body has developed from scattered simple perithecia, and as a



result all intermediate variations are to be found. This term must, therefore, designate a type of structure towards which the course of development tends, rather than any sharply definite type of organization.

A *fruiting area* will be considered as including the entire area of the substratum in which perithecia or compound fruit bodies are produced.

The perithecia within a fruiting area may be scattered or clustered. There are varying degrees of the grouping of the perithecia, but these can be indicated.

There may, or may not, be a development of entostromatic mycelium about the scattered or clustered perithecia, within a fruiting area.

If an entostromatic mycelium is developed about the perithecia, such an entostromatic area may or may not be surrounded by a darkened marginal zone.

The entostromatic area is often lighter in color than the surrounding bark tissue, on account of the development of a hyaline mycelium within this area, or the darkening of the disintegrated bark tissues outside this area, or both. Such an area will be designated as a *differentiated entostromatic area*.

The ostioles of the perithecia within a fruiting area may be *separately erumpent* or *collectively erumpent*. Clustered perithecia, or the perithecia within an isolated stroma, as in *Diatrype*, may have separately erumpent ostioles.

An entostromatic area (stroma) will be spoken of as *effused* when it contains numerous separately erumpent perithecia, or more than one cluster of perithecia either separately or collectively erumpent; and as *isolated* when it contains only one cluster of perithecia, either collectively or separately erumpent. The differentiation between isolated and effused stromata, in such cases as the genus *Diatrype*, must remain purely arbitrary, as it depends merely upon the areal expansion of the stroma.

The *disc* (*Placodium* of Ruhland) is that portion of the stroma which is erumpent through the periderm or epidermis. Such a disc is not always present, for in some species the perithecial neck may burst through the periderm by means of the mechanical pressure of its own growth, or may grow upwards as a mass of hyphae which push their way through the periderm by means of their disintegrating action. This disc may be composed of ectostromatic tissue (*ectoplacodialer* type of Ruhland, as in *Diaporthe*), or of entostromatic tissue (*entoplacodialer* type of Ruhland, as in *Diatrype*). When the disc is ectostromatic, it may consist of a sharply differentiated conical stroma, or it may be an irregular pulvinate mass, so intimately intergrown with a well developed entostroma within the surface bark layers that no sharp distinction can be drawn between the two tissues.

The ostioles, which are merely the erumpent portion of the perithecial necks (*Tubulus* of Füsting), may vary greatly in length in certain species, depending upon the moisture conditions. The tips of the ostioles may be

*punctate*, that is, merely perforated by the circular opening of the central canal; or they may be *sulcate*, that is, have 3-5 clefts or grooves in the apex of the wall of the central canal.

The size and shape of the perithecium is of diagnostic value only in the occasional separation of species. The perithecia may be arranged parallel to one another and perpendicular to the surface; they may converge towards, or be arranged *circinately* (in a circle) about, a central disc; they may be *monostichous* (in a single layer), or *polystichous* (in two or more irregular layers); or they may be imbedded in a variously developed stroma, or in the unaltered bark cortex.

The work of recent European mycologists, von Höhnelt in particular, has brought out the importance of what they have termed the perithecial "Nukleus," in determining broad general relationships. This term is used to include the totality of structures within the perithecial wall. The application of the term *Nukleus* to this aggregate of structures is ambiguous and very misleading. In the following discussion the term *perithecial centrum* will be used instead. This perithecial centrum includes the asci, paraphyses, and the inner portion of the perithecial wall (subhymenium).

The spores themselves provide one of the most nearly constant and dependable characters that we have. Objections have been raised to the over-emphasis upon spore characters. The consideration of spore characters alone of course gives rise to the same sort of errors to which the exclusive use of any one character leads. Similar spore forms have arisen in different lines of evolution, and correlated characters must be used to determine their true relationships. Some species are slow in attaining the final coloration or septation of their spores. Certain species of *Pseudovalsa*, for instance, do not show the coloration of their spores until they are fully mature. The consideration of immature spores of such species has also led to confusion. The variations in any given species can be determined more easily, however, in the case of spore characters than in that of almost any other character, since the variations are usually characteristic for a species and not due to environmental conditions. The spores may be uniseriate or biseriate in the ascus, may possess various types of appendages or gelatinous envelopes, may be hyaline or colored, variously septate, and of various shapes. Spore size is valuable chiefly as a specific character.

The structure of the asci and their arrangement in the hymenial layer have been shown by von Höhnelt to be important characters in the separation of large related groups. The asci may be 4-, 8-, or many-spored. They may have more or less elongated persistent stalks and form a definite hymenial layer; or their stalks may be delicate and evanescent, and the asci may lie irregularly massed in the perithecium. The asci may be clavate to cylindrical, with variously thickened tips or pores.

The presence or absence of paraphyses has been much used in the separation of species, genera, and even families. Wherever this character has

en used as a major character, confusion has arisen. It is rather the rule than the exception to encounter contrary statements by different authors to presence or absence of paraphyses in any given species. The paraphysis-like structures in the Sphaeriales are of the "pseudo-paraphysis" or "metaphysis" type of Petrak (92, pp. 65, 68). A consideration of the character and development of these structures easily explains the situation. They arise as numerous delicate, filiform hyphae; they appear before the asci, and apparently act as a nurse tissue to the developing asci. In many species they are entirely used up, or absorbed, by the growth of the asci; in other species delicate remnants persist in the mature perithecium; while in still other forms these paraphyses are more numerous and persistent, and appear in large numbers in the mature perithecium. There is a gradual increase in the number and persistence of the paraphyses as one passes from the simpler to the higher forms, in certain developmental series. There are two facts, however, which make the use of these structures as diagnostic characters unreliable. In the first place, their number and visibility vary with the degree of maturity of the perithecium. Secondly, the paraphyses, when only sparsely present, are, on account of their delicacy and transparency, difficult to distinguish even under the best microscopic definition. As a taxonomic character, therefore, they should be used only in correlation with other characters.

#### TAXONOMY

The taxonomy of the ascomycetes is at present one of confusion. On account of our inadequate knowledge of the life-history connections of the spore forms of these fungi, the separate classification of the imperfect spore forms, as form genera, will probably always remain as the only convenient and practical means of their identification. The chaotic condition of the classification of the fungi imperfecti need not be discussed here; it is self-evident.

The taxonomic literature of the Sphaeriales is riddled with inaccuracy and confusion. It is this chaotic condition of the synonymy which has apparently discouraged any comparative study of the group. The old systems of Fuckel and Nitschke still stand with very few improvements. One need make only a superficial examination of the pages of Saccardo's "Sylloge" to be impressed by the numerous inadequate and even inaccurate descriptions of species and genera. Any herbarium will show inaccurately determined exsiccata and immature or half-decayed material; and many a species has been described from such an impossible remnant. The scattered condition of the literature containing specific descriptions should discourage the description of new species, but the result is apparently the opposite, judging from the multiplication of species and the resulting duplication. It is apparently easier to describe a specimen as a new species than to identify it. Until the recent publications of von Höhnelt, Sydow, Theissen, Petrak, and other European mycologists, no comparative study, or effort to sift or

arrange this mass of scattered and often worthless material and data, had been undertaken. These workers have straightened out much of the synonymy and morphology of numerous European species, and have pointed out many of the broader relationships.

This later work, however, has emphasized minute structural differences without a proper appreciation of the variation of such structures within a given species. The generic concept has likewise become narrowed. There seems to be a general practice at present to erect new genera for every slight variation from the type species. This method employed by a number of separate workers, with various viewpoints, leads to a large number of monotypic genera. This tendency is apparently due to two causes. In the first place, the limits of the old genera are indistinct; in the second place, we have no fixed ideas as to the relationships within the larger groups. Most of the characters of the sphaeriaceous fungi seem to vary independently of one another, and the tendency to erect a new genus for each different grouping of characters will result in an enormous number of artificial genera, each containing one or a few species. On the other hand, genera separated on the basis of the correlated characters of a developmental series must necessarily have arbitrarily chosen limits. This is true because of the numerous transitional species in any developmental series. The position of such transitional species, however, is always definitely fixed in the phylogenetic scheme, no matter what arbitrary generic lines are chosen. Whatever method of separation is used, however, genera should not be erected in a haphazard manner upon one or a few species without a thorough knowledge of the relationships and variations of the other species of the group in which the genus lies. The present confusion of newly erected, recently abandoned, vaguely distinct, and often overlapping genera speaks eloquently against such a practice.

The first step, as the writer sees it, is to attack each large natural group as a whole. Luckily the preliminary step of collecting, collating, and assimilating a large amount of data has to a large extent been done by the European workers just mentioned.

The imperfect stage has long been used to obtain evidence for relationships in the Sphaeriales. Nitschke and Winter have used the type of conidial fruit body as a character in the separation of the Melanconideae and Melogrammaceae. The genera *Scoptria*, *Melanconis*, and many others have been separated more or less upon the basis of the imperfect stage. Recently Klebahn (72) and Wollenweber (134) have used the imperfect stage as a means of separating genera. Von Höhnelt and Petrak have also used this method in erecting new genera. The conclusions of these two workers have been drawn, however, from observational associations in a comparatively small number of species, rather than from cultural connections of a large number of related forms. With the increasing tendency to consider the entire life history of these fungi in determining their classifica-

tion, it becomes more and more essential to have a number of authentic connections of perfect and imperfect stages, in order to determine the relationships of species and genera.

On account of these facts, and because the writer considers that a study of the complete life histories and morphological development of a large number of forms is necessary to an understanding of the phylogeny of any group, single-spore or single-ascus isolations have been made of a large number of species of the group under consideration, and their life histories have been worked out in culture. Detailed accounts of most of these studies have been published in separate papers (127, 128, 129, 130), or are now in press. The following forms have been studied in culture.

#### SPECIES CULTIVATED

##### Life histories published:

1. *Diaporthe oncostoma* (Duby) Fck.
2. *Quaternaria Persoonii* Tul.
3. *Diatrype virescens* (Schw.) E. & E.
4. *Diatrypella Frostii* Pk.
5. *Valsaria exasperans* (Ger.) E. & E.
6. *Valsaria insitiva* Ces. & de Not.
7. *Diaporthe furfuracea* (Fr.) Sacc.
8. *Diaporthe albovelata* (B. & C.) Sacc.
9. *Valsa Kunzei* Fr.
10. *Diaporthe binoculata* (Ell.) Sacc.

##### Life histories in press:

11. *Diatrype stigma* (Hoffm.) de Not.
12. *Cryptosphaeria populina* (Pers.) Sacc.
13. *Cryptosphaeria eunomia* (Fr.) Grev.
14. *Eutypella cerviculata* (Fr.) Sacc.
15. *Diaporthe galericulata* (Tul.) Sacc.
16. *Diaporthe obscura* (Pk.) Sacc.
17. *Eutypella tumida* (E. & E.) comb. nov.
18. *Eutypella fraxinicola* (Cke. & Pk.) Sacc.
19. *Cryptovalsa Nitschkei* Fck.
20. *Cryptovalsa* sp.
21. *Diatrypella quercina* (Pers.) Nit. f. *crataegi* E. & E.
22. *Diatrypella favacea* (Fr.) Nit.

##### Life histories not yet published:

23. *Diaporthe marginalis* Pk.
24. *Pseudovalsa longipes* (Tul.) Sacc.
25. *Diaporthe faginea* (Curr.) Sacc.
26. *Diaporthe pruni* E. & E.
27. *Diaporthe* sp.
28. *Cryptospora cinctula* (Cke. & Pk.) Sacc.
29. *Cryptospora* sp.

## THE PHYLOGENY OF THE STROMATIC SPHAERIALES

It is the intention of the following discussion, then, to consider in some detail the relationships that may be made evident by a consideration of the stromatic forms of the Sphaeriales, chiefly of the families Valsaceae, Melanconidaceae, Melogrammataceae, and Diatrypaceae, from the various viewpoints just discussed.

In the discussion of the imperfect stages, stress will be laid on the morphological and developmental similarities. As the nomenclature of this group is confused and misleading, the position and synonymy of these forms will be considered only in a general way.

The four families of the Sphaeriales just mentioned fall naturally into two large classes, each of which is characterized by a number of correlated characters. Von Höhnelt (59, 60) recognized the existence of these two large groups of forms in the Sphaeriales, and designated them as the "Allantosphaeriaceen" and the "Diaporthen." He based his separation of these two families upon the internal structure of the perithecium, which he termed the perithecial "Nukleus" and which is here spoken of as the perithecial centrum. We shall see as we proceed with the discussion of these two groups that there are other characters, in both the perfect and the imperfect stages of the life histories of these fungi, which are correlated with this difference in the perithecial centrum.

## Allantosphaeriaceae

In his family of the "Allantosphaeriaceen," von Höhnelt (56, p. 54; 60, p. 127) included four subfamilies, which he named the Diatrypeen v. H. (non Aut.), Calosphaeriaceen v. H., Valseen v. H., and Coronophoreen v. H. His Diatrypaceae and Valsaceae are the two families of particular interest in this discussion. The Calosphaeriaceae and Coronophoraceae are chiefly non-stromatic forms, and have not been studied by the writer. The Valsaceae, although resembling the Diatrypaceae in the allantoid character of their spores, differ in almost every other point and in reality belong with the Diaporthaceae. Von Höhnelt himself recognized this fact, for he said: "Die Valseen sind allantoidsporigen Diaporthen und werden daher in dem endgültigen System der Sphaeriaceen zu denselben gebracht werden müssen, da mir der Bau des Nukleus wichtiger erscheint als die Sporenform" (60, p. 129). Our discussion here, therefore, narrows down to his Diatrypaceae, which contain, besides the old genera *Diatrype*, *Quaternaria*, and *Diatrypella*, all the subgenera of the genus *Valsa* Nit. except *Euvalsa* and *Leucostoma*. The genus *Endoxyla* also belongs in this group. In his discussion of the "allantoidsporigen Sphaeriaceen" (60), von Höhnelt excluded *Endoxyla*, having previously placed it with the genus *Anthostoma* (53). In a later paper (63), however, he recognized *Endoxyla* as an allantoid-spored form. The genera *Anthostoma* and *Valsaria*, although not having allantoid spores, show many other characters of this group, and will be discussed under this heading in this paper.

The first striking resemblance common to the members of this group is the structure of the perithecial centrum, which was thoroughly investigated and recognized by von Höhnelt. All these forms, except the genera *Valsaria* and *Anthostoma*, have allantoid spores (in *Anthostoma*, the spores are inequilateral or slightly curved, and in some species of *Valsaria* they are curved). The spores are also always more or less colored, ranging from a dilute yellowish hyaline in some species of *Eutypa* to a dark brown in *Anthostoma*. They are usually one-celled, but become septate in a few genera, as in *Endoxyla*. The spores are biseriate in the ascus (becoming obliquely uniseriate, or uniseriate in *Anthostoma* and *Valsaria*). In contrast, the genera *Euvalsa* and *Leucostoma* have purely hyaline spores. The structure and arrangement of the asci is also characteristic in the *Allantosphaeriaceae*. The asci are 8- or many-spored, clavate, often with a thickened tip in some genera, always more or less equally long-stipitate, and arranged in a definite hymenial layer covering the base and sides of the perithecium. This occurrence of more or less persistent ascus stalks of equal length gives rise to the regular more or less colored layer of spore-bearing asci about the margin of the perithecial cavity, so characteristic of this group. Paraphyses are typically present, becoming gelatinous in age. The ostioles of the group are characteristically sulcate. The evolution of the stroma in this group shows that the entostroma has first arisen as a broadly effused tissue and has later become limited in area, resulting in isolated stromatic patches. Each of the series to be discussed shows transitional variations from the effused to the isolated type of stroma. The stroma, therefore, can not be used to separate genera but is of value in tracing the development within a series, when used in connection with other correlated characters. *Hypoxylon*, *Diatrype*, *Anthostoma*, and *Diaporthe* in the traditional sense, for instance, all include both effused and isolated types of stromata in various species.

Another very constant character in this group is the imperfect stage. The conidia, called spermatia by the earlier writers, are always more or less elongated-cylindrical to filiform, hyaline, one-celled, and more or less bent or hamate. These conidia are formed in enclosed locules or in more or less exposed layers, either in ectostromata on the bark surface or in the entostroma within the bark cortex. Some of the more primitive effused forms also produce conidia on free conidiophores. These variations in the configuration of the hymenium and stroma may be found within the same species (especially in culture), as well as between species.

From the preceding discussion we see that von Höhnelt's "*Allantosphaeriaceen*," including the genus *Endoxyla*, but exclusive of his "*Valseen*," form a compact and related group of species, with a number of correlated characters in common. It is reasonable to assume that a number of species showing so many characters in common, in both stages of their life history, form a phylogenetically related group. Such an assumption is strengthened

by the fact that there can be traced within this group four parallel series of species, which arise from the more primitive forms and show the gradual evolution of the stroma and the isolated compound fruit body. These series differ from one another in the type of stromatic development, as well as in certain correlated morphological characters.

Let us now turn to a consideration of the relationships within these series.

*Eutypa and Cryptosphaeria*

Since the origin of the stromatic Sphaeriales from the simple Sphaeriales is characterized by the development of a stromatic tissue in connection with the perithecial fruit bodies, it is natural to suppose that this character will be the one which will be the most plastic and will show the greatest variation. One would not expect the development of such a character, due probably to a change in life habit such as the formation of immersed perithecia, to be a monophyletic one. It would be more logical to suppose that such a development began in a number of separate species with immersed perithecia, belonging to widely separated genera. The existence of a stromatic tissue about the fruit bodies of species with widely varying spore and perithecial characters supports this view.

Assuming such a polyphyletic origin, we should expect that the main lines of stromatic development in any large group, such as the Allantosphaeriaceae, would arise from a group of species with a primitive type of stromatic development but a comparatively wide range of variation in other associated characters. This is precisely the case. The effused stroma, or protostromatic type of Ruhland, is usually considered as the more primitive form and most closely related to the simple Sphaeriales. This by no means should imply, however, that all effused stromata are of a primitive type. The effused *Diatrypes* and *Hypoxylons*, for instance, show an advanced type of stromatic development. The taxonomy of the effused forms is in a confused condition. The genera *Eutypa*, *Cryptosphaeria*, *Cryptovalsa*, *Diatrype*, *Endoxyla*, and *Anthostoma* all contain effused forms. This means in other words that all these genera can be derived from forms with a primitive type of stroma.

The species of the genera *Eutypa* and *Cryptosphaeria*, here to be considered, are probably the most primitive of these species with an effused stroma.

Greville's (39, no. 201) conception of the genus *Cryptosphaeria* was very broad, including even pycnidial forms which are immersed in the substratum. The subsequent descriptions of the genus have been exceedingly brief, and in no way delimit it from the Tulasnes' (123, p. 52) description of the genus *Eutypa*. The only existing difference seems to be that all species of *Cryptosphaeria* are immersed in the bark, while most of the species of *Eutypa* inhabit decorticated wood. Many species of *Eutypa*, however, are described which inhabit the bark cortex, and many of these



species can undoubtedly exist in either condition. As the species of these two genera are also often confused with the lower forms of the genera *Diatrype* and *Eutypella*, it will be necessary here to disregard more or less the present taxonomic position of these species and to seek for relationships instead.

In our later discussions of the *Allantosphaeriaceae*, we shall have occasion to differentiate between those species which do, and those which do not, form conidial locules in the entostromatic tissue of the bark. Although too little is known as yet of the species of these two genera to make any sharp differentiation in this respect, yet there seem to be certain groups of forms which may serve as possible examples of the origin of these conditions.

The most primitive types of the genus *Eutypa* are probably represented by such forms as *Eutypa Acharii* Tul., *E. lata* (Pers.) Tul., and *E. spinosa* (Pers.) Tul., which grow on decorticated wood. These forms seem to represent the origin of the types with an ectostromatic conidial fruit body. The perithecia of these species are rather evenly scattered just within the wood. There is a very sparse development of entostromatic mycelium, but pockets, or scattered hyphae, of a black-walled mycelium are often found within the wood. As a result, these stromata are not raised above the surface of the substratum. The surface of the substratum is more or less blackened by the growth within the surface layers of the wood tissue of dark-walled hyphae, which blacken and disintegrate the cells and form a blackened crust. This blackening is the only evidence of a stroma. These dark-colored hyphae may give rise to a hyphomycetous conidial stage, consisting of free, septate, brown conidiophores bearing hyaline curved conidia. This formation of brownish hyphae in the surface layers of the substratum, and the occurrence of a hyphomycetous stage, are characteristic of many simple *Sphaeriales*, certain superficial wood-inhabiting forms in particular.

Other species of this primitive type, as some specimens of *Eutypa lata* (Syd. Myc. Germ. no. 234), show a richer development of mycelium within the wood, as a result of which the stromatic areas become somewhat raised above the general surface of the substratum. This indicates the beginning of entostromatic development. The surface of the stroma is, however, always covered by a blackened crust of the substratum.

Two types of imperfect stages have been described for the species of *Eutypa* just discussed. The first of these consists of a hyphomycetous stage of free, septate, brown conidiophores, bearing clusters of filiform, curved, hyaline, one-celled conidia. This hyphomycetous stage, as already mentioned, shows the close relationship between these species and the simple *Sphaeriales*. Such free conidiophores have been figured by the Tulasnes (123, Pl. VII, figs. 9, 10) for *E. Acharii*, and have been described by Füsting (36, p. 193) for *E. lata*. Brefeld (14, pp. 238, 239) grew several species of *Eutypa*, and obtained a free conidiophore stage of *E. subsecta* Fr. but failed to obtain this stage of *E. Acharii* in culture.

The second type of imperfect stage in these species consists of a conidial hymenium formed in or on an ectostromatic cushion. These hymenial layers may be in enclosed locules, open cavities, or exposed layers, but are always formed in or on a stromatic cushion arising on the surface of the entostroma. The Tulasnes (123, Pl. VII, figs. 8, 9, 11) figure such a conidial stage for *E. Acharii*, and Füsting (36, p. 193) describes similar ones for *E. Acharii* and *E. lata*. Brefeld (14, p. 239) obtained such conidial stromata in cultures of *E. Acharii*.

This formation of the conidial hymenium in ectostromatic cushions is most important, for it is the development of this ectostromatic tissue which characterizes the *Diatrype* series, as will be seen later. Füsting (36, p. 193) states that ectostromata are formed by *E. lata* and *E. flavovirescens* only when they grow on bark, and that, when growing on decorticated wood, *E. lata* seldom develops sufficient ectostromatic tissue to produce conidial tissue. This would support the view, which the writer here puts forward, that the development of the ectostroma has resulted from the formation of the perithecia within the bark, and from the concomitant necessity of producing some sort of mechanical tissue for the rupture of the periderm.

The second type of *Eutypa*-like forms to which the writer wishes to call attention includes the species of *Cryptosphaeria* and such forms as *E. flavovirescens* (Hoffm.) Sacc., and *Diatrype Hochelagae* E. & E. The chief differences between these forms and those previously mentioned are the greater development of the entostromatic tissue and the formation of the conidial locules within this tissue.

*Eutypa flavovirescens* (Hoffm.) Sacc. shows a rather special development and possesses characters which suggest its intermediacy between the two groups of species here considered. It may be found growing either on decorticated wood or in bark. The stromatic tissue, which is developed beneath the surface layers of the substratum cells, is very strongly developed and yellow-green in color. The perithecia are formed in this tissue, which is composed almost entirely of fungous hyphae and forms a raised stromatic area. On wood it is covered by several layers of the wood cells, but on bark it bursts open the periderm. The conidial locules of this species are formed within the entostromatic tissue, within which the perithecia arise. The Tulasnes (123, Pl. VII, figs. 3, 4) figure this imperfect stage, and it has been described by Füsting (36, p. 193). Brefeld (14, p. 239) grew *E. flavovirescens* and obtained free conidiophores which often united to form *Coremia*-like structures. The formation of free conidiophores shows that this species, in spite of its well developed entostroma, is closely related to the more primitive forms of the preceding group.

*Diatrype Hochelagae* E. & E., which is not a *Diatrype* but a *Eutypa*, has a structure very similar to *E. flavovirescens*, with the exception that the entostromatic mycelium is hyaline instead of yellow-green. It occurs on decorticated twigs of *Ulmus*, and is very similar in structure to *Diatrype*

(*Eutypella*) *tumida* E. & E., found on bark of the same host. It therefore represents a type from which the species of *Eutypella* have probably arisen.

The species of *Cryptosphaeria* show a more advanced type of stromatic structure than the species already mentioned. *Cryptosphaeria populina* (Pers.) Sacc. may be taken as an example of this genus. The writer has carried this species in culture, and a more complete account of its development may be found in a previous paper (129). This species grows in the bark of various species of *Populus* and forms a distinctly differentiated entostromatic area, outlined by a zone of blackened tissue. There is formed, on the surface of the bark beneath the periderm, a layer of closely interwoven and heavily blackened hyphae. This might be considered ectostromatic tissue, but is more likely homologous to the blackened hyphae in the surface layers of the substratum of such species as *E. Acharii*, etc., since it does not function as either a mechanical or a spore-bearing tissue. The important points to keep in mind in connection with this species are the lack of any hyaline ectostromatic tissue, the entire lack of any rupturing of the periderm, the separate emergence of the perithecial necks through the periderm, and the presence of numerous filiform paraphyses in the perithecium. These characters, we shall later see, suggest this species as a type giving rise to the fourth and last series of the Allantosphaeriaceae.

The imperfect stage of this species was not obtained in either agar or twig cultures.<sup>3</sup> Saccardo (100, vol. 3, p. 602) gives *Cytosporina myriocarpa* Sacc. as the imperfect stage of *Cryptosphaeria myriocarpa* (Wint.) Sacc. This *Cytosporina* is described as forming chambered locules beneath the periderm in the entostroma. This entostromatic origin of the conidial locules is probably characteristic of the genus *Cryptosphaeria*. All the conidia of this second group of *Eutypa*-like forms, so far as they are known, are of the typical filiform, hyaline, curved character.

It is such forms as those just named which represent the logical origin of those two series of the Allantosphaeriaceae that show the formation of the conidial locules in the entostromatic tissue of the bark. Correlated with this character is the lesser development of ectostromatic tissue in these series.

The two groups of species in question are taken as examples of a large number of forms with a primitive type of stroma which show numerous variations. Our knowledge of these forms, and particularly of their development, is very incomplete, but they undoubtedly compose the group of species from which all the lines of development within the Allantosphaeriaceae diverge.

<sup>3</sup> The imperfect stage of *Cryptosphaeria populina* has recently been obtained in culture by the writer on twigs of *Populus grandidentata* Michx. which had been coated with paraffin. The conidia formed were filiform, hyaline, and curved, and measured  $17-21 \times 1-2 \mu$ . They were produced in ellipsoid locules within a stromatic tissue, which arose within and beneath the periderm. This stromatic formation was abnormal, for perithecia were produced in this same tissue. No definite conclusions can therefore be reached as to the ecto- or ento-

*First Series: Diatrype*

This first series includes the species of the genus *Diatrype* possessing either an effused or an isolated stroma. This series is characterized by the formation of a strongly developed deciduous ectostroma, which throws off the periderm from the entire surface of the equally well developed entostroma, whose darkened surface layers then form the widely erumpent disc. This strong development of the entostroma gives a characteristically distinct and abrupt margin to the strongly erumpent stroma. The perithecia are usually arranged parallel to one another in a single layer; they have short, stout necks and are separately erumpent through the encrusted surface of the entostroma as sulcate, disc-like ostioles. The conidia are always formed from ectostromatic tissue. The hymenial layers may be formed in enclosed locules, or in more or less open cavities. The conidia are long-filiform, one-celled, strongly curved, and hyaline.

We have seen that *Eutypa Acharii* possesses ectostromatic cushions in which such conidial cavities are formed, and it is from such primitive forms that the species of this series have probably arisen. At the present time there are no types of development known intermediate between that of *E. Acharii* and that of *Diatrype stigma*, which shows a rather advanced stromatic structure. If the development of some of the numerous transitional species of *Eutypa* were worked out, however, they would undoubtedly reveal such intermediate steps in the development of the stroma.

The Tulasnes (123, Pl. VI) described and figured *Diatrype stigma* under the name of *Stictosphaeria Hoffmanni* Tul. Füsting (36) studied the development of this species from field material. The writer has carried this species in pure culture and has published an account of its life history and development in a previous paper (129). The stroma of this species, although effused, shows an advanced type of development. The perithecia are imbedded in a strongly differentiated entostroma, bounded by a blackened marginal zone. There is early developed, on the bark surface, an effused layer of ectostroma which swells rapidly at maturity and throws off the periderm over large areas. This exposes the developing entostroma, whose surface then rapidly becomes blackened, forming the erumpent disc. The young ectostroma increases greatly in thickness at various points, and in these cushions of tissue labyrinthiform conidial locules are formed. Füsting (36) describes conidia of three sizes ( $24-30 \times 0.5 \mu$ ,  $10-18 (20-30) \times 1 \mu$ , and  $6 \times 1 \mu$ ). The Tulasnes (123, p. 50) mention only the small allantoid type, measuring  $4-6 \times 1 \mu$ . The writer found only one type of conidia in his cultures. These were crescent-shaped, hyaline, and measured  $9-10 \times 1-1.5 \mu$ . It is possible, of course, that there are several closely related species with different conidia.

The distinction between effused and isolated stromata in the genus *Diatrype* is purely an arbitrary one, based upon the areal expansion of the stroma. Some specimens of *D. stigma* itself show stromata of much more restricted extent than other specimens of the same species.

*Diatrype bullata* (Hoffm.) Fr. illustrates the further reduction of the area of the individual stroma. The stromata of this species are only lightly raised above the substratum, and are only 2-5 mm. in diameter.

The distinctly isolated type of stroma, as found in *Diatrype albopruinosa* Schw.) Cke., *D. virescens* (Schw.) E. & E., *D. disciformis* (Hoffm.) Fr., *D. tremellephora* Ell., and many others, is usually 1-3 mm. in diameter. These isolated stromata have the same type of development as has *D. stigma*, except that it is confined to a smaller area.

In his discussion of the development of the stroma of *D. disciformis*, Ruhland (99) states that a strongly developed ectostroma is formed which is later thrown off by the development of the entostroma beneath. This same behavior is shown in a striking manner by Ellis' specimen of *D. tremellephora* (N. A. F. no. 775) on *Magnolia glauca*. The strongly developed ectostroma of this species breaks through the thick periderm and appears on the surface as a small reddish "tremelloid" disc, which swells greatly upon being moistened. This ectostroma is later thrown off by the entostroma, which then becomes the erumpent disc.

In these forms with an isolated stroma the entostroma is very strongly developed and is composed almost entirely of fungous hyphae. As a result the stroma is strongly erumpent and sharply defined. We shall see later that structures very similar to these *Diatrype* stromata have arisen in other evolutionary series. None of these, however, shows the deciduous ectostroma, and they are arrived at by a different type of development.

The imperfect stages of these isolated forms are also ectostromatic. The ectostroma above developing perithecia often remains sterile, but a conidial hymenium may be formed in open cavities upon the sides or flanks of such a conidial ectostroma. Such a conidial formation is described by Ruhland (99) for *D. disciformis*. Where ectostromatic growth is rapid and no perithecia arise beneath this tissue, labyrinthiform locules may be produced within these ectostromata. The upper portion of the stroma is often used up in conidium-formation, and the locules become exposed as open cavities. Brefeld (14) grew *D. disciformis* and *D. bullata* and obtained in both cases mycelial cushions, within which were formed irregularly enclosed conidial locules.

The writer has carried *Diatrype virescens* in pure culture and obtained various types of conidial fructifications. On agar these were irregular cavities formed within a superficial mycelial mass. On beech twigs under dry conditions they consisted of a basal stroma, often with a dome-shaped central portion. The surface of this stroma was covered with a conidial hymenium (127, Pl. XXII, fig. C, 5). Under moist conditions on beech twigs the ectostromatic cushion developed extensively and formed an erumpent superficial stroma, with a hymenium on its surface and within irregularly exposed cavities sunken in the stroma (127, Pl. XXII, fig. C, 6). This variability is characteristic of these ectostromatic conidial fruit bodies.

It can be seen from what has been said that the genus *Diatrype* in itself presents a distinct line of development from the effused to the isolated type of stroma. This phylogenetic series is different from all others of this family in the formation of a deciduous ectostroma, which exposes the darkened surface of the entostroma as a widely erumpent disc.

### *Quaternaria*

This genus is rather ill defined, and some of its species undoubtedly belong to other genera. Two species have been studied by the writer. One of these, *Q. Morthieri* Fck., is, as Winter (132, p. 826) suggests, a species of *Anthostoma*. The second species, *Q. Persoonii* Tul., which is the type species and characteristic of the genus, has been carried in pure culture by the writer, and its life history has been published (127). This species does not belong in the same series as the genus *Diatrype*, but occupies an isolated position and shows a peculiar combination of characters representative of several of the series of the *Allantosphaeriaceae*. It is discussed here on account of the ectostromatic character of its conidial stage.

The perithecia of this species are in small groups of 2-4 within the upper bark tissues. The lower bark tissues and wood surface are blackened. There is a development of entostromatic mycelium about the perithecial groups, giving them a pustulate appearance. This mycelium often develops over extensive areas, forming an effused pustulate stroma in which numerous clusters of perithecia are developed. These perithecia are collectively erumpent through a small ectostromatic disc. This effused stroma is often bounded beneath by a definite blackened zone.

In this species the grouping of the perithecia has taken place before the entostroma is sharply differentiated and while it still forms effused patches. It differs from such bark-inhabiting species of *Eutypa* as *E. ludibunda*, which may have clustered perithecia, only in the somewhat more strongly developed entostroma and in the blackening of the lower bark tissues. The formation of the clustered perithecia within an effused entostroma is typical of the simpler forms of stroma found in the genera *Eutypella* and *Cryptovalsa*. Although this species shows only a weakly developed ectostroma and has its perithecia collectively erumpent, it possesses an imperfect stage similar to that of the species of *Diatrype*.

This imperfect stage is given by the Tulasnes (123, p. 105) as *Naemospora crocea* (Pers.) Moug. & Nest., or *Libertella faginea* Maz., and is figured (Pl. XII, figs. 18, 19) as having elliptical or irregular cavities within an ectostroma. In the writer's cultures on twigs, the conidial stroma was always ectostromatic. Practically the entire stroma was used up in spore-formation, leaving an open cavity within a slight basal stroma. There sometimes remained a central dome-shaped projection of this basal portion of the stroma (127, Pl. XXI, fig. B, 4). The conidia are filiform, hyaline, curved, and  $13-20 \times 0.5-1 \mu$ .

This species therefore shows a stromatic structure intermediate between *Eutypa* and *Eutypella*, but with a conidial stage similar to that found in *Diatrype*. Forms similar to this might easily have given rise to the following series by an increase in the number of spores formed in the ascus.

### *Second Series*

The most outstanding character of this second series is the polysporous condition of the asci. This polysporous condition has apparently arisen as the result of an increase in the number of divisions of the nuclei in the ascus, and therefore is derived from the 8-spored condition. Petrak (94, p. 227) has claimed that the species of the genus *Valsella* with polysporous asci are not even specifically distinct from certain species of the subgenus *Leucostoma* which have 8-spored asci. Sydow's *Myc. Germ.* no. 2132, of *Valsa translucens* (de Not.) Ces. & de Not., is an example of this identity, showing both the 8-spored and the many-spored asci in separate stromata of identical structure.

The two genera, *Cryptovalsa* and *Diatrypella*, of this series are, however, distinct from the 8-spored forms. There are two possibilities as to the origin of this polysporous group of species. Either they have a common origin and show a development of their own, or they have arisen from a number of different species or genera by the increase in the number of spores in the ascus. At first glance the latter seems to be the case, for there are species which superficially resemble both *Eutypella* and *Diatrype*. A detailed examination of the two genera shows, however, that they consist of a series of species showing a gradual development from the effused to the isolated type of stroma. The individuals of this series, furthermore, show a closer relationship to one another than to any of the 8-spored genera. The polysporous forms are considered here, therefore, as a monophyletic series.

This polysporous series presents a type of development intermediate between that of *Diatrype* and that of *Eutypella*. The structure of the entostroma is that of the *Eutypella* series, but the conidial stage is usually ectostromatic, and in that respect similar to that of the *Diatrype* series. As pointed out under the discussion of *Quaternaria Persoonii*, this series can be conceived as originating from forms similar to that species.

### *Cryptovalsa*

This genus includes the species with the more primitive type of stromatic development and is only arbitrarily separated from *Diatrypella* upon the basis of its more effused stroma.

The most primitive polysporous type observed by the writer is one collected on *Cercis canadensis* L. This species has the same spore and ascus measurements as *Cryptovalsa sparsa* E. & E., but shows a different stromatic structure. The perithecia are scattered singly or in pairs; they arise in the

bark tissues, but become partially or almost wholly sunken in the wood at maturity. Just above the perithecial initials there occurs a blackening of the bark tissues. Some of the hyphae in this region penetrate into the periderm and weaken that tissue. The growing perithecial necks penetrate the periderm at these weakened points. The blackened zone extends downward to the wood surface, where it spreads out between the perithecia in the lower bark layers. There is very little entostromatic development and practically no ectostroma, and the dorsal blackened zone is the only evidence of a stroma.

This species shows the occurrence of the polysporous condition of the ascus at a level even lower than that of *Quaternaria*.

*Cryptovalsa sparsa* E. & E. has a somewhat more advanced type of stroma, and resembles *Quaternaria Persoonii* in many respects. The entostroma is weakly developed, usually effused, and outlined at its margin by a blackened zone. The lower bark tissues are often strongly blackened and disintegrated. The perithecia occur singly or in small clusters of 2 to 4. Practically no ectostroma is produced above the perithecial clusters.

*Cryptovalsa Nitschkei* Fck. was carried in pure culture by the writer, and its development has been described in a previous paper (130). This species has a more strongly differentiated entostroma than *C. sparsa*. The entostromatic areas are usually effused and contain a number of perithecial clusters, although isolated stromata occasionally occur with only one group of perithecia. The marginal zone is here sharply defined. In this species, as in the two preceding, there is merely a slight development of blackened hyphae in the surface bark layers above the perithecia. Some of these hyphae penetrate the periderm and weaken it sufficiently to allow the perithecial necks to rupture this tissue. It is interesting to note that in culture, however, this species produced well developed erumpent ectostromata which were yellow in color. This formation of ectostromata we shall find occurring normally in the higher members of this series. In culture the conidia were formed in labyrinthiform locules within the ectostromata. There was sometimes a strong development of entostromatic mycelium in the surface layers of the bark cells just beneath the ectostromata. These two tissues often formed a more or less homogeneous mass, giving the appearance of an ectostroma partially sunken in the bark. When this was the case, the conidial locules often extended into the entostromatic portion of this tissue. Such a fusion of the ectostromatic mycelium with an entostromatic mycelium in the surface layers of the bark, and a consequent extension of the conidial locules into the entostromatic portion of this tissue, will be found to be characteristic of this series.

### *Diatrypella*

Many species placed in the genus *Diatrypella* have a structure almost identical with that of certain species of *Cryptovalsa*, while others show a



distinctly isolated type of stroma. Three species of this genus have been studied in culture by the writer. We shall first consider a series of species of this genus all of which produce a yellowish-green ectostroma; a character we saw appearing in the cultures of *Cryptovalsa Nitschkei*.

The first species of this series is *Diatrypella quercina* (Pers.) Nit. forma *crataegi* E. & E. (N. A. F. 2d ser., no. 2741). The developmental history of this species has been published by the writer elsewhere (130). This species shows a slightly more advanced type of development than that found in *Cryptovalsa Nitschkei*. The differentiated entostromatic areas are usually effused and contain a number of perithecial clusters. The perithecia are developed beneath small ectostromatic cushions of a yellow-green color. There is often a rich development of entostromatic mycelium beneath these ectostromata, and the fusion of these two tissues gives the appearance of an ectostroma partially sunken in the bark tissue. This is similar to the condition described in *Cryptovalsa Nitschkei*, but it occurs here in nature as well as in culture. The conidial stage consists of an irregular locule in the ectostroma, or occasionally extending into the adjacent entostromatic tissue.

*Diatrypella Frostii* Pk., which shows another slight advance in stromatic structure, has also been grown by the writer (127). In this species the entostromatic areas are usually isolated, containing only a single group of perithecia. Occasional areas are found, however, with two or more perithecial clusters. A yellow-green ectostromatic disc is usually found above each perithecial cluster. A yellow-green entostromatic mycelium is also produced about the perithecia. In culture there was often no sharp distinction between the ectostroma and the entostroma of the surface bark layers. The conidia were produced in locules within the ectostroma; they were filiform, curved, hyaline, and  $30-50 \times 1 \mu$ .

*Diatrypella betulina* Pk. represents the climax type of this series. This species is most interesting in that it presents a structure which is superficially identical with that of a *Diatrype*, but which has arisen in a different manner. This species has a widely erumpent blackish disc, containing separately erumpent, quadrisulcate, disc-like ostioles. In vertical section the disc tissue is seen to consist entirely of yellowish-green fungous tissue with a blackened surface crust. This greenish tissue originates, however, upon the bark surface beneath the periderm, and is therefore ectostromatic. The ectostroma is, therefore, not deciduous in this case, but forms the erumpent disc itself. The perithecia arise within a hyaline entostromatic tissue in the surface bark layers. These perithecia increase enormously in diameter (to  $700-800 \mu$ ), and in so doing push up the well developed ectostroma and give rise to the strongly erumpent stroma.

The possession in common of a yellowish-green ectostroma by the species of the preceding series might be considered sufficient to place them in a separate group. As this is the only differential character, however, a

thorough investigation of all the polysporous species is necessary before such a separation is made. The following forms have a hyaline ectostroma.

*Diatrypella prominens* (Howe) E. & E. has isolated entostromatic areas. The hyaline ectostromata are only weakly developed, but the entostromatic mycelium about the perithecia is strongly developed, resulting in a strongly pustulate but only slightly erumpent stroma.

*Diatrypella favacea* (Fr.) Nit. has been carried in culture by the writer. The details of its development are given in a previous paper (130). The stromata are of the isolated type. The ectostroma is weakly developed and lies chiefly within the periderm itself. The entostroma is strongly developed and causes the pustulate character of the stroma. The ectostroma is not sharply differentiated from the upper entostromatic tissue. The conidial locules are formed within the ectostroma, or sometimes extend into the entostromatic tissue of the upper bark. The conidia are hyaline, curved, and  $18-40 \times 1-1.5 \mu$ .

*Diatrype nigro-annulata* (Grev.), *D. Tocciaeana* de Not., and *D. verruciformis* (Ehr.) Nit. are all similar in structure to the species just mentioned.

*Diatrypella discoidea* Cke. & Pk. is another species which imitates a *Diatrype* in appearance. The stromatic structure is similar to that of *D. betulina*, except that the disc tissue is hyaline instead of greenish. In this case, however, remnants of bark cells are found within the disc tissue, and it must therefore be considered as entostromatic in origin. Unfortunately, young stages were not available to the writer for a study of the development of this species. If a well developed deciduous ectostroma is formed in this species, it would be a true *Diatrype* with polysporous asci.

In the preceding discussion of the series with polysporous asci, we have seen that the lower forms of *Cryptovalsa* have a very simple structure. The entostroma is weakly developed, the perithecia are scattered, and practically no ectostroma is formed. As we advanced from the lower to the higher forms, we found a rapid increase in entostromatic development, and a more gradual increase in the formation of ectostromatic tissue. In the genus *Diatrypella* the entostromata become isolated in character. Besides the polysporous condition of the asci, the series is characterized by the fusion of a weakly developed ectostroma with the entostromatic tissue of the upper bark layers. The conidial locules are formed in this tissue, and, although primarily ectostromatic, often extend slightly into the entostromatic tissue below.

The weakly developed ectostroma and the strongly developed entostroma give rise to the characteristic external appearance of the species of this group; most of the species have a strongly pustulate stroma, but a closely adherent periderm and a weakly erumpent disc. Both Füsting (36, p. 186) and Ruhland (99) noticed this correlation between the appearance and the stromatic development of the genus *Diatrypella* and cited this as the differential character between *Diatrypella* and *Diatrype*. The same

appearance and development, on the other hand, are typical of the next series to be discussed.

The climax types of this series, such as *D. betulina* and *D. discoidea*, however, have very well developed ectostromata and widely erumpent discs. This fact was recognized, to some extent, by Ruhland (99, p. 21), who states that *D. quercina* (Pers.) Nit. approaches *Diatrype* in its strongly developed ectostroma.

### *Third Series: Eutypella*

This series includes the single genus *Eutypella*. It is characterized by the 8-spored asci, the small but distinctly differentiated ectostroma, and the conidial locules formed within the entostromatic tissue of the bark. The stromatic configuration in this genus varies from forms with an effused entostroma and more or less scattered perithecia to those with definitely isolated stromata.

*Eutypella tumida* (E. & E.) Weh. is interesting in this connection in showing almost this entire range of variation within one species. This species was described by Ellis as *Diatrype tumida*. Its life history, as worked out in pure culture by the writer (130), has shown, however, that it is a typical *Eutypella* and not a *Diatrype*. The entostromatic areas are usually effused and contain numerous perithecial clusters or separate perithecia; these areas may in some cases be restricted in area and contain only a single group of perithecia. Numerous small conical ectostromata are formed on the surface of the bark in the effused areas. The perithecia arise singly or in small clusters beneath these ectostromata. The variation in the number and proximity of these ectostromata, and in the number and size of the perithecia formed beneath them, gives rise to a wide range of variation in the outward appearance of the stromatic pustules. In the writer's cultures, the pycnidial locules were sometimes formed within the ectostromata and sometimes in the entostroma of the bark. In nature, the ectostromatic growth not being stimulated as it is in moist culture tubes, the labyrinthiform conidial locules are always formed within the entostromatic tissue of the bark.

*Eutypella platani* (Schw.) Sacc., *E. fraxinicola* (Cke. & Pk.) Sacc., and other species have a structure very similar to that of the species last named. *E. fraxinicola* has been carried in culture by the writer. Its development is similar to that of *E. tumida* and is described in detail elsewhere (130). Where large erumpent ectostromata were formed in culture, conidial locules developed in this tissue. The imperfect fruit bodies were usually formed in the entostroma, however, and consisted of numerous locules which coalesced to form labyrinthiform chambers. The conidial stage found in nature was also of this entostromatic type.

Other species of *Eutypella* show typical isolated stromata. *E. cerviculata* (Fr.) Sacc., which the writer has studied in culture (129), is an example of this group. The ectostroma of this species is developed largely within the

periderm tissue, the cells of which are split apart over a rather wide area. As a result of this type of growth, a large, rather broadly erumpent stroma is produced, containing many perithecia and a large cluster of well developed sulcate ostioles. In culture the conidial locules were formed within both the ectostroma and the entostroma. The conidia were crescent-shaped, hyaline, and  $11-15 \times 1 \mu$ . Saccardo (100, vol. 3, p. 602) gives a conidial stage (*Cytosporina cerviculata* Sacc.) similar to this, but with conidia  $20-22 \times 0.75 \mu$ , as belonging to *E. cerviculata*.

*Eutypella angulosa* (Nit.) Sacc., *E. alnifraga* (Wahl.) Sacc., *E. similis* (Karst.) Sacc., *E. prunastri* (Pers.) Sacc., and *E. sorbi* (Alb. & Schw.) Sacc. are all similar to, if not identical with, the species last named.

All the species of *Eutypella* thus far mentioned have spores  $6-12 \mu$  in length. There is another group of species of this genus which have very small spores ( $3-5 \times 1.5 \mu$ ) and asci ( $21-24 \times 4 \mu$ ). The ostioles of these species tend to be long-cylindrical, sinuous, and almost hair-like, especially when growing under moist conditions. Included in this group are such species as *Eutypella fici* E. & E., *E. capillata* E. & E., *E. scoparia* (Schw.), *E. glandulosa* (Cke.) Ell., *E. microcarpa* E. & E., and *E. exigua* E. & E.

*E. fici* and *E. glandulosa* have isolated perithecial clusters, but there is no differentiated entostromatic area and the blackened zone is present merely as a continuous blackened crust on the surface of the bark. In *E. scoparia*, on the other hand, there is a well differentiated entostroma, and the marginal zone dips deeply into the bark, cutting off isolated or somewhat effused areas. The writer has examined only three of these species, and it is possible that an examination of other forms would show a developmental series within this group.

The imperfect stage of the genus *Eutypella* is very similar in all cases so far known. It falls in the form genus *Cytosporina*, and from the preceding examples we see that it consists of numerous or labyrinthiform locules formed in the entostromatic masses of mycelium which arise within the bark tissues. Saccardo (100, vol. 3, pp. 602, 603) gives *Cytosporina stellulata* Sacc. and *C. ailanthis* Sacc. as the imperfect stages of *Eutypella stellulata* (Fr.) Sacc. and *E. ailanthis* Sacc., respectively. The Tulasnes (123, p. 187) describe two types of pycnidia for *E. sorbi* (Alb. & Schw.) Sacc. One of these forms, *Cytospora rubescens* Fr., possesses allantoid conidia  $3.5 \mu$  in length, while the other has the typical filiform conidia,  $35-45 \mu$  long. Brefeld (14, p. 240) suggests a relationship between *Eutypella* and *Euvalsa* on the basis of this occurrence of a second allantoid type of conidium in *E. sorbi*. We have seen, however, that *Euvalsa* differs from *Eutypella* in almost every other character. Brefeld failed to obtain fruit bodies of either *Eutypella sorbi* or *E. prunastri* (Pers.) Sacc. in culture.

In his key to the Diatrypaceae, von Höhnelt (60, p. 128) separates the genera *Diatrype* and *Eutypella* on the basis of their imperfect stages; he gives *Diatrype* as having *Libertella*, and *Eutypella* as having *Cytosporina*,

for the conidial stage. We have seen, however, that there are other correlated differential characters in the perithecial stroma.

The structure and the evolutionary development of the stroma in this *Eutypella* series are very similar to what is found in the previously mentioned series with polysporous asci. This series differs, however, in the 8-spored asci, and in the formation of the conidial locules completely within the entostromatic tissues of the bark.

#### *Fourth Series*

This series is more heterogeneous than any thus far described. This is true because of the variation of several non-stromatic characters and of the comparatively few transitional species. The apparent relationship of some of the forms here included (*Anthostoma*) to certain of the simple *Sphaeriales* (*Rosellinia*, *Anthostomella*) also makes certain of the suggestions in the following outline more or less tentative. A further study of the life histories and development of a number of these species is needed either to confirm or to alter these suggestions.

The structure of the perithecial centrum in all the species included here is, however, typical of the *Allantosphaeriaceae* (certain species of *Valsaria*, to be considered later, are exceptions to this statement). There are also certain definite evolutionary tendencies in this group. The spores, for instance, show tendencies to become dark-brown, straight-cylindrical or inequilaterally ellipsoid, and uniseriately arranged in the ascus. The asci likewise become cylindrical and shorter-stalked, and there is an increase in the number of filiform hyaline paraphyses. The stromatic development of this series is almost entirely entostromatic, showing an almost entire reduction of the ectostroma.

#### *Cryptosphaeria*

Several species of this genus show the origin of brown cylindrical ascospores. As has already been pointed out, the only tissue in *C. populina* which can be considered as ectostroma is a blackened sclerotial layer on the surface of the bark. Paraphyses are rather abundantly present in its perithecia, but the spores are still yellowish-hyaline, allantoid, and biseriate in the ascus. It shows in other words the main features, but none of the variations, of this series.

*Cryptosphaeria vicinula* (Nyl.) Karst. shows the next step in the development of this series. This species has the same stromatic structure as *C. populina*, but the stromata are more strongly raised and show a tendency to break up into smaller patches. The spores of this species are brown, one-celled, nearly straight-cylindrical or slightly curved, and somewhat larger than those of *C. populina*; they are irregularly biseriate to uniseriate in the ascus.

A specimen of this species on *Alnus*, examined by the writer, shows

stromata less strongly raised above the substratum, and in still more restricted areas. The spores of this specimen were also larger and straighter than those on *Populus*, showing a further development of this tendency. These two forms show the transition from the yellow-hyaline, allantoid, biserial condition of the ascospores to the dark-brown, straight-cylindrical, uniserial type. Let us now examine several species which show the tendency towards the septation of the ascospore.

*Cryptosphaeria millepunctata* Grev. (*Endoxyla fraxini* E. & E.) is a species with an effused entostroma which possesses septate ascospores. This species has been carried in culture by the writer, and its life history is given in a previous paper (129). The entostroma is widely effused, occupying the entire upper portion of the bark tissue over wide areas; the lower bark tissues are heavily blackened. In nature no ectostroma is formed. There is only a slight blackening of the bark tissue about each of the separately erumpent perithecial necks. The conidial locules, in nature, are labyrinthiform, and formed with the perithecia in the entostromatic tissue of the bark. In culture, ectostromatic cushions were formed on the bark surface and conidial locules were produced within these stromata. The conidia were filiform, curved, hyaline, and  $30-40 \times 1 \mu$ . A second, shorter type of conidium,  $13-20 \times 1 \mu$ , was formed in twig cultures. The spores of this species are quite variable; they are hyaline, allantoid, and one-celled when young, but become dark brown, 1- to 4-septate, and nearly straight cylindrical at maturity. They are biserial in the ascus. Brefeld (14) grew this species and noted that the spores became 1- to 3-septate upon germination. The writer has also observed the non-septate spores become 1- to 5-septate, and constricted at the septa upon germination. Brefeld reported the formation in culture of free conidiophores on stromatic cushions, as well as the production of conidial locules within these stromata.

This species shows the occurrence of septate spores in a member of this series with an effused stroma. The formation of conidial locules in the entostroma is characteristic of all the known imperfect stages of this series.

### *Valsaria*

The relationships of this genus are difficult to determine. As it stands today it contains species belonging to both the Allantosphaeriaceae and the Diaporthaceae.

There are two species, the structure of whose perithecial centrum is that of the Allantosphaeriaceae. The spores of these two species are undoubtedly derived from the allantoid type, and their stromatic development is similar to that of *Anthostoma*.

The first of these species, *Valsaria moroides* (Cke. & Pk.) Sacc., has a strongly pustulate stroma, but the disc is scarcely erumpent. Isolated, differentiated, entostromatic areas are formed, but these are not bounded by any dark marginal zone. There is no ectostroma formed, and the

rupturing of the periderm takes place in a manner characteristic of both *Valsaria* and *Anthostoma*. There is a blackening of the hyphae in the surface layers of the bark, and some of these hyphae penetrate into the periderm and weaken this tissue. The growth of the perithecial necks and of this mass of entostroma bursts open the periderm at the point weakened by the penetration of the hyphae mentioned. The spores of this species are variable; they are irregularly biseriate to uniseriate in the ascus, either straight-cylindrical or allantoid, brown in color, contain 2-6 yellowish areas, and are uniseptate at maturity. Both the straight-cylindrical and the allantoid spores are constricted at the septum.

The second species with spores of an allantoid character is *Valsaria allantospora* E. & E. (31, p. 343) (*V. coloradensis* E. & E.; 32, p. 342). *V. moroides* var. *aceris* Rehm (U. S. Dept. Agr. Myc. and Path. Coll. no. 1687) is an immature specimen of this species. The entostromatic area in this case is light in color, often somewhat effused, and bounded by a definite blackened zone. The rupturing of the periderm is similar to that in the preceding species. The spores are biseriate, dark brown, and vary from the allantoid non-septate to the straight-cylindrical septate condition; they are not constricted at the septum.

Unfortunately, the writer has been unable to obtain the two last-named species in culture, and their imperfect stage is apparently unknown. The spores of these species show a clear relationship to the allantoid forms. The remaining species of the genus, which should be considered the true *Valsarias*, have broad elliptical-fusoid spores; they are dark brown, uniseptate, constricted at the septum, and are not at all characteristic of the *Allantosphaeriaceae*.

The position of these species is somewhat doubtful. Such species as *Valsaria insitiva* Ces. & de Not. and *V. exasperans* (Ger.) E. & E. show many characters similar to those of the genus *Anthostoma*. A close examination of the perithecial centrum and of the character of the spores, however, indicates their relationship to be with the *Diaporthaceae*, probably near *Endothia*. In those species of the *Diaporthaceae* which have numerous persistent paraphyses, the asci are held within this mass of paraphyses and appear as a definite, persistent, hymenial layer, similar to that formed in the *Allantosphaeriaceae*. This is the case in these species of *Valsaria*. The attachment of the asci in these species, however, is seen to be by a short, stout, somewhat curved base, which is characteristic of the *Diaporthaceae*, while in *V. moroides* and *V. allantospora* the base of the ascus consists of a short but narrowly tapered stipe, which is typical of the *Allantosphaeriaceae*.

The method of rupturing the periderm is similar in *V. insitiva* and *V. exasperans* to that in the rest of the species of this series. In *V. insitiva* there is a rapid proliferation of black-walled hyphae in the upper bark layers. The bark cells are absorbed by, or become imbedded within, this tissue, which ruptures the periderm and forms an erumpent disc. This is

a purely mechanical tissue, for many such discs are formed which have no perithecia formed beneath them. A blackened zone extends downward from this disc and surrounds the perithecial cluster.

In *V. exasperans* an area of the upper bark tissues is early cut off by a darkened marginal zone. A rapid proliferation of entostromatic hyphae takes place within this enclosing zone. The perithecia arise within this tissue, and its subsequent growth produces a large erumpent stroma, composed largely of grayish-hyaline hyphae, within which the remnants of the bark cells are imbedded.

From what has been said we see that these two *Valsarias* have a purely entostromatic development similar to that found in *V. moroides* and *V. allantospora*.

Both *V. insitiva* and *V. exasperans* have been grown by the writer, and their life histories have been published (127). As usual, in culture the growth was superficial, and large erumpent stromata were produced which were apparently ectostromatic. In both species the conidial locules were labyrinthiform, and were formed in such stromata. In nature the imperfect stage of *V. insitiva* was found in the same entostroma with the perithecia. In *V. exasperans* the conidial stage occurred in separate entostromata, identical with the perithecial stromata. The conidial locules are therefore entostromatic in these species also. The conidia, from culture, were minute, elliptical, one-celled, and hyaline. These conidia differ from any so far found in the Allantosphaeriaceae. The Tulasnes (123, Pl. XI, figs. 13, 16) figure a similar conidial stage for *V. rubricosa* (Fr.) Sacc. Ruhland (99) studied the development of *V. rubricosa*. In spite of the fact that he distinctly states that the stroma of this species arises as a proliferation of hyphae within the bark, he places it in his "haplostromatic" series, which type of stroma he in turn considers as being entirely ectostromatic. This illustrates the confused notion of these two types of stromatic tissue held even by Ruhland himself.

From our discussion of the genus *Valsaria*, it seems that, although it has the stromatic development of an *Anthostoma*, it is in reality more closely related to *Endothia*. This may be considered as another case of an analogous type of structure arising in two widely separated series. We have emphasized here the similarities between *Valsaria* and the other forms of this series. Following the discussion of *Endothia* we shall consider its relationship to that genus.

#### *Anthostoma*

We now come to the genus *Anthostoma*, which is characterized by its one-celled, brown, cylindrical or inequilateral ascospores. We have seen that certain species of *Cryptosphaeria* show a transition from the allantoid yellow-hyaline ascospore to the brown cylindrical type. Such transitional forms, as well as other characters soon to be discussed, suggest the origin of the *Anthostoma* group from allantoid-spored forms. Such a hypothesis



would unify the entire group of the Allantosphaeriaceae. It would overlook entirely, however, another fact, namely, that there is a long series of forms extending back into the simple Sphaeriales which show an identical spore form, and which seem undoubtedly related to Anthostoma. The reference is to such genera as Anthostomella and Rosellinia. The entire family of the Xylariaceae, furthermore, is characterized by the same spore form and can be considered a further development of the Anthostoma type. In fact, this evidence is so convincing that it seems probable that such a series might constitute a third large group, comparable to the two here considered under the stromatic Sphaeriales. Petrak (94, p. 253) has already suggested the relationships of these forms. Very little is known of the life histories and development of the lower forms of this hypothetical series, however, and such knowledge must first be obtained before any conclusions can be drawn. Let us first consider what evidence we have.

The genus Anthostoma was divided by Nitschke (83) into two subgenera, Euanthostoma and Lopadostoma. The subgenus Euanthostoma consists of effused forms, which usually inhabit decorticated wood. Such forms are closely related to the simpler forms found in Anthostomella, where the perithecia are more scattered, and with a separate blackened "clypeus" about each perithecial neck. Unfortunately, material of these species has not been available to the writer, either for culture or for study. Their relationships, therefore, must be omitted in the present discussion.

*Anthostoma decipiens* (DC.) Nit. is an effused form, which was described and figured by the Tulasnes (123, Pl. VIII, figs. 1-8) as an Eutypa. This species is figured as having labyrinthiform conidial locules formed within the entostroma; the conidia are hyaline and allantoid. Free conidiophores bearing similar conidia are also figured. This imperfect stage is strikingly like those previously noted in the genus Eutypa.

Petrak (94, p. 254) separated the subgenus Lopadostoma into two groups. He has made this separation of the entire subgenus, apparently, upon the study of two species only. He gives *A. turgidum* (Fr.) Nit. as an example of the first group, and *A. gastrinum* (Fr.) Sacc. as typical of the second group. *A. microsporum* Karst., which he also mentions, he places in neither group. The only character for the basis of such a separation that the writer can see is the presence or absence of the dark marginal zone.

*Anthostoma turgidum* (Fr.) Nit., which typifies Petrak's first group without a marginal zone, has a stromatic structure similar to that of *Quaternaria Persoonii*. The small perithecial clusters are scattered in the light-colored upper layers of the bark, while the lower bark layers are blackened and disintegrated. The periderm is ruptured by the mechanical force of the growth of the fused ostioles.

*Diatrype dryophilum* Curr. var. *minor* Grev. (N. A. F. no. 87), which was later recognized by Ellis (29, p. 581) as an Anthostoma, also belongs in this group. The periderm is ruptured by the massed ostioles, and the surface of the bark is blackened, but there is no bounding zone in the bark.

*Anthostoma microsporum* Karst. shows a well differentiated entostroma of blackened tissue, but no black marginal zone.

*Anthostoma gastrinum*, characteristic of the second group, has light-colored entostromatic areas, which are often somewhat effused and are always bounded by a darkened zone. The periderm is ruptured by the growth of a mass of blackened tissue, composed of the fused perithecial necks and an entostromatic tissue formed in the surface bark layers.

*Diatrype phaeosperma* Ell. [*Anthostoma phaeosperma* (Ell.) Sacc.] (N. A. F. no. 1557) is a form with isolated entostromatic areas and a blackened bounding zone. The superficial appearance of the stroma of this species is that of a *Diatrype*. Its internal structure is identical with that described for *Diatrypella discoidea*; that is, the disc tissue is composed of a well developed entostromatic tissue with a darkened superficial crust. In the case of this species, an examination of young stages has shown that no ectostroma is formed and that the development of the entostroma itself ruptures the periderm. The spores of this species, although dark-brown, are slightly but distinctly allantoid. In this respect this species is similar to *Quaternaria Morthieri*, which we have already seen should in reality be placed in the genus *Anthostoma*. *Q. Morthieri* has a stromatic structure almost identical with that of *A. turgidum* and would fall in the first group.

*Anthostoma juglandinum* Rehm var. *caryae* Rehm (98) also belongs in this second group. It has effused entostromatic areas, with a marginal zone. The rupture of the periderm is similar to that found in the rest of the genus.

The conidial connections of the subgenus *Lopadostoma* are not very well known. Nitschke (83, p. 121) states that the conidial stromata of *A. turgidum* are Cytospora-like, containing numerous locules in which are formed allantoid, hyaline conidia,  $10 \times 1.5 \mu$ . Brefeld (14, pp. 241, 242) grew *A. turgidum* and *A. Xylostei* (Pers.), but obtained only sterile mycelium. Fuckel gave *Myxosporium sanguineum* as the conidial stage of *A. gastrinum*, but Winter (132, p. 759) considers this doubtful.

From the preceding discussion it seems certain that many species of *Anthostoma* have arisen from forms with allantoid spores. *A. decipiens* shows the hyphomycetous stage characteristic of the genus *Eutypa*, as well as the entostromatic pycnidial stage typical of so many of the allantoid-spored genera. *A. phaeosperma* and *Quaternaria (Anthostoma) Morthieri*, as we have seen, have distinctly allantoid spores. *A. turgidum* has been reported as having a conidial stage typical of the *Allantosphaeriaceae*.

How, then, can we harmonize these facts with the existence of a continuous series of forms, from *Rosellinia* through *Anthostomella* and, supposedly, *Anthostoma* to *Hypoxylon* and the *Xylariaceae*? There are two possibilities. Either there are two separate series, or the *Allantosphaeriaceae* have arisen from the series of forms with brown cylindrical ascospores. The writer hesitates to make this latter suggestion, because it involves the

origin of light-colored spores from darker-colored ones, which is the reverse of what generally seems to be true of the evolution of the spores in other groups. This view is particularly tempting because of the lack of any definite series of allantoid-spored forms in the simple Sphaeriales, from which the Allantosphaeriaceae could be derived. This does not explain the fact, however, that the lighter-colored spores usually occur in those species of the Allantosphaeriaceae with the simpler stromatic structure. It is the writer's opinion, rather, that further study of these forms will reveal two groups of brown-spored forms. One of these will consist of the Rosellinia—Anthostomella—Xylariaceae series; and the second will consist of forms related to, and derived from, the Allantosphaeriaceae. Only a further study of the life histories of a number of these species will determine what differential characters can be used to distinguish these two groups.

#### *Summary (Allantosphaeriaceae)*

Let us now review briefly the phylogenetic development of the Allantosphaeriaceae as outlined above. We found the simplest forms to be species of *Eutypa*, living chiefly upon decorticated wood. The perithecia of these forms are scattered singly, but are united by a blackened crust on the surface of the substratum. A primitive hyphomycetous stage is retained in their life history, but they also form conidial locules within a stroma.

The first step in the evolution of these forms was the production of a rich mycelium about the fruit bodies, within the substratum. This soon resulted in the development of a differentiated entostromatic area, bounded by a blackened marginal zone. At this stage in the evolution of the group, we can already discern the four separate lines of development already discussed, since there are effused forms with separately erumpent perithecia in all these series.

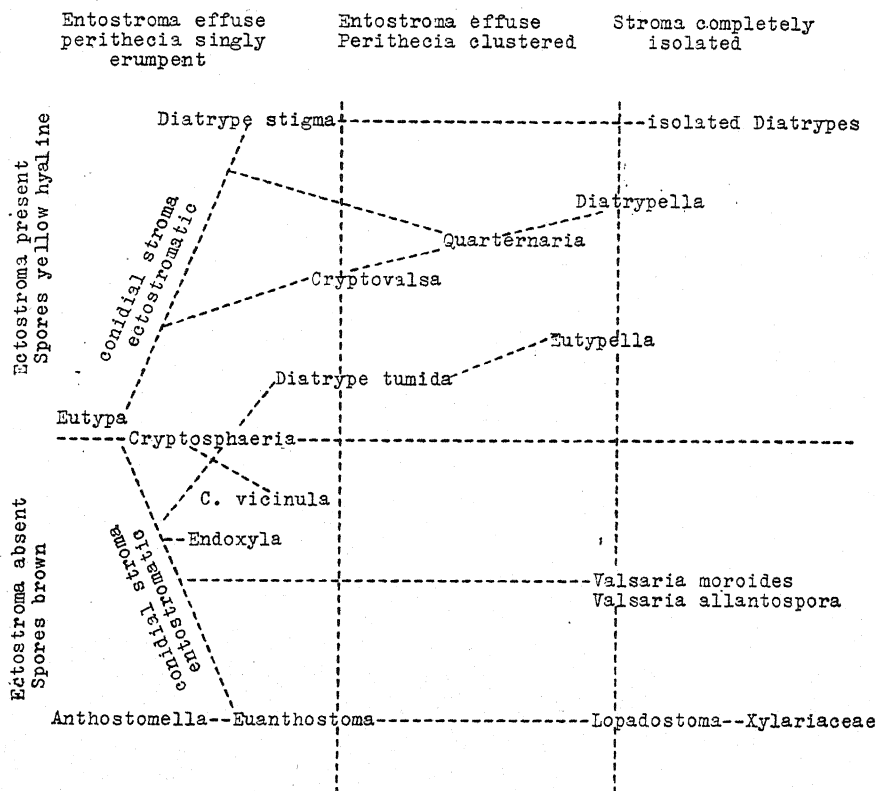
One group of forms (*Diatrype*) has produced, very early in its evolutionary development, a very strongly developed and deciduous ectostroma. The result of the efficient casting off of the periderm by this deciduous mechanical tissue is a characteristic structure. In the first place, the conidial locules are formed within this well developed ectostromatic tissue; in the second place, the perithecial necks, having no resistant tissue to penetrate, remain separately erumpent. Even in the most isolated and well developed stromata of this series, as in *D. virescens* or *D. disciformis*, there is no convergence of the perithecial necks. In this respect this series stands in contrast to all the rest of the family.

In the remaining series there occurs a grouping of the perithecia and a convergence of the perithecial necks, resulting in collectively erumpent ostioles. This clustering of the perithecia takes place along with the development of the entostroma, and we often find numerous perithecial clusters formed within a single effused entostroma. At the same time that this grouping of the perithecia takes place, there is developed above each

group an ectostromatic cushion, or some other type of mechanical tissue to aid in the rupture of the periderm. As soon as the clustering of the perithecia has taken place and a compound fruit body is organized, the development of the entostromatic tissue becomes limited to the area about each cluster of perithecia, and there results a true isolated type of stroma.

Such an evolution of the stroma and the compound fruit body can be traced in that series of forms which have the polysporous ascus as a constant character. In this series there is practically no ectostroma formed in the lower forms, such as *Cryptovalsa sparsa*, but in the higher isolated types of stroma, such as *Diatrypella betulina* and *D. quercina*, this tissue becomes rather strongly developed. The conidial stage of this series is formed primarily in the ectostroma, but, on account of the well developed entostroma in the surface bark layers, the conidial locules may extend somewhat into this tissue.

The two genera *Cryptovalsa* and *Diatrypella* are merely arbitrarily selected portions of the same developmental series. Some species of this group, we have seen, differ from the rest in the yellow-green coloration of the ectostroma.



TEXT FIG. 1. The phylogenetic relationships of the Allantosphaeriaceae.

The genus *Eutypella* has a stromatic development like that of the preceding series. It differs in the 8-spored asci and in the conidial stage, which is here formed primarily within the entostroma although locules may also be occasionally found in the ectostroma.

The fourth and last series is characterized by its entostromatic conidial stage and by the entostromatic character of the tissue which functions in the rupture of the periderm. This series shows the most complete reduction of the ectostroma and the most strongly developed entostroma. In the genus *Cryptosphaeria* there is a tendency toward a coloration, a uniseriate arrangement of the spores, and the formation of numerous paraphyses. In *C. millepunctata*, *Valsaria moroides*, and *V. allantospora* there is a tendency toward both a coloration and a septation of the ascospores, as well as an increase in the number of paraphyses. In the genus *Anthostoma* we find a number of species of a climax type, with brown, cylindrical, uniseriate spores, cylindrical, short-stalked asci, and numerous paraphyses. This genus suggests a relationship with the series of brown-spored forms already mentioned, and needs further study to determine its exact relationships.

The phylogenetic relationships of this family of the Allantosphaeriaceae may be represented diagrammatically as in text figure 1.

### Diaporthaceae

The second large group of the stromatic Sphaeriales which shows a common relationship is that belonging to the general type designated by von Höhnelt (59) as the "Diaporthen." The "Diaporthen" are also characterized by the structure of their perithecial centrum. In this group the asci have no definite stalk and do not remain in a definite hymenial layer after maturity, as in the Allantosphaeriaceae. When moistened, the base of the ascus contracts and dissolves, pinching off the ascus from its attachment. The ascus walls are delicate, and break down in water. As a result of these qualities of the ascus, the entire central cavity of the perithecium becomes filled with a mass of free asci and spores, giving the characteristic appearance of this type of perithecial centrum. In many species there is a ring or collar of protoplasm which extends into the thickened apical wall of the ascus, such as is found in *Gnomonia* and related genera. The paraphyses are delicate and numerous in the young perithecium, but are very evanescent. In the mature perithecium they are either absent, or present as a few delicate, broad, band-like filaments. In the higher forms, however, the paraphyses become more numerous, persistent, and filamentous. In these cases the asci may be held in place by the mass of paraphyses, and the appearance of the perithecial centrum is then somewhat different from that of the lower forms. Petrak (92, pp. 65, 68) has designated the band-like evanescent paraphyses as "pseudoparaphyses" and the filamentous persistent structures of the higher forms as "metaphyses."

The spores of this group are various in shape and septation, being elliptical, allantoid, or long-cylindrical, hyaline to colored, and 1- to many-septate.

The imperfect stage is always formed in, or on, an ectostromatic cushion, which in some cases may be intimately associated with a well developed entostroma beneath. The conidia may be formed in enclosed locules, open cavities, or exposed layers. Most of the species have two types of conidia. One type is one-celled, hyaline, and rod-shaped to allantoid or long-cylindrical. The second type varies from hyaline to brown and from one- to many-celled. Three series of development will be considered in this group.

#### *First Series*

The first series of related species which we shall attempt to trace in the Diaporthaceae includes the genera *Diaporthe*, *Melanconis*, and *Pseudovalsa*. Starting with the primitive forms, we soon encounter what seem to be two separate lines of phylogenetic development, one of which again seems separable into two groups. These three series culminate in the three genera just mentioned.

The presence or absence of a dark marginal zone about the perithecia in this series seems to be correlated with the type of imperfect fruit body formed. Petrak (94, p. 320) noted that the absence of such a dark marginal zone distinguishes the genus *Melanconis*, with dark-colored conidia, from the genus *Diaporthe*, which has hyaline conidia. There seem to be, however, certain species, as for example those in Petrak's (89, p. 117) genus *Cryptodiaporthe*, which lack a dark zone and yet have hyaline conidia. These facts can be established only by further cultural studies. For convenience, however, the species without such a marginal blackened zone will be spoken of as the *Melanconis* group, while those possessing such a zone will be spoken of as the *Diaporthe* group.

The evolution within this group shows the gradual development of a number of characters along several diverging lines. There is a general tendency throughout towards the development of a stroma and the clustering of the perithecia. There is also an increase in the number and persistence of the paraphysis-like structures, although the data concerning this character are still somewhat vague and conflicting. The spores in the lower forms are all 2-celled and hyaline. In the *Diaporthe* group the spores remain hyaline; in the genus *Melanconis* the spores become brown in the higher forms; while in *Pseudovalsa* they become brown and many-celled. In most species the spores are biseriate in the ascus, but in some species of *Melanconis* they become uniseriate. In the *Diaporthe* group the conidia are hyaline and non-septate, and are formed within pycnidial locules; in the *Melanconis* group the conidia tend to become colored and septate, and the hymenial layers tend to become more exposed. In the genus *Melanconis* the conidia remain non-septate and brown, while in the genus *Pseudovalsa* they become septate and brown.

*Diaporthe*

The genus *Diaporthe* includes an unusually large number of named species, many of which are undoubtedly synonymous. There remain, however, a large number of species within a comparatively small range of variation, resulting in almost every conceivable combination of characters. The only constant character is the 2-celled hyaline spore. If we add to this character the presence of a dark marginal line and of a *Phomopsis* stage in the life history, we delimit a compact, related group which can ultimately represent the natural outlines of the genus *Diaporthe*.

The origin of the simpler forms of the genus *Diaporthe* from the genus *Gnomonia* is so evident that it is unnecessary to go into detail in this respect. Species such as *D. rostellata* (Fr.) Nit. resemble a typical *Gnomonia* in every way, and have been placed in the genus *Diaporthe* only because of their stem-inhabiting character. The distinction, previously mentioned, between the *Diaporthe* group and the *Melanconis* group of this series can not be traced with any assurance in the lower forms. In certain *Euporthe* forms, as for example, *D. exercitalis* (Pk.) Sacc., the only evidence of a stroma is that furnished by the thickly scattered perithecia, while others with similarly scattered perithecia show, in addition, a blackening of the surface of the substratum. Further connections of perfect and imperfect stages are needed to show any relation of these forms to the two groups mentioned.

The compound fruit bodies in this genus have arisen as the result of two tendencies: the development of a stromatic mycelium, and the clustering of the perithecia. Nitschke's (83) division of the genus into the three subgenera *Euporthe*, *Tetrastaga*, and *Chorostate* draws arbitrary lines directly across these two tendencies and, as von Höhnelt (57, p. 382) has remarked, is an unnatural division. A single species may show a variation over the range of all three subgenera. *D. fasciculata* Nit. in the subgenus *Euporthe*, and *D. oncostoma* (Duby) Fck. in the subgenus *Chorostate*, for instance, are identical. The position of the perithecia within the wood tissue (*Euporthe*) in many species inhabiting herbaceous stems is merely due to a lack of sufficient bark cortex for these fruit bodies. This is shown by the fact that where a sufficient cortex is developed (as noted by the writer in the cases of *D. euspina* (Cke. & Ell.) Sacc. on *Chenopodium* and *D. aculeata* (Schw.) Sacc. on *Phytolacca*) the perithecia may develop partially or entirely within this tissue.

The development of the stroma and the clustering of the perithecia take place independently in the various species. As already noted, the first appearance of the stroma is a blackening of the surface of the substratum, as seen in *D. arctii* (Lasch.) Nit., *D. euspina* (Cke. & Ell.) Sacc., etc., which show no clustering of the perithecia. In other species, as *D. densissima* Ell. and *D. salicella* (Fr.) Sacc., there is a tendency toward a clustering of the perithecia, or at least a convergence of the necks of neighboring perithecia

so as to become collectively erumpent, without any evidence of a marginal zone. Such species show a possible origin of the *Melanconis* group. Petrak (90, p. 180) gives the imperfect stage of *D. salicella* as *Discella carbonacea* (Fr.) Berk. & Br., which he considers a *Septomyxa*, and which has 2-celled hyaline conidia. This connection was by observation only, and the same *Discella* was previously given by Petrak as the imperfect stage of *Diaporthe tessella* on the same basis. Such information is of course usable only as a suggestion until verified by culture, but if true such an imperfect stage would be characteristic rather of those forms of the *Melanconis* group related to *Pseudovalsa*, as will be brought out later.

Such species as *D. Woolworthii*<sup>4</sup> (Pk.) Sacc. and *D. ulmicola* E. & E. show a clustering of the perithecia and also a darkened zone on the surface of the bark tissues. *D. ulmicola* also shows the beginning of the formation of an entostromatic mycelium within the bark tissue. In the *Diaporthe* group, as this development of an entostromatic mycelium increases in amount, there is usually formed a differentiated entostromatic area, which is surrounded by the characteristic blackened marginal line.

There are a few species which develop such a differentiated entostromatic area and retain the separately erumpent character of the perithecia. *D. decedens* (Fr.) Fck. shows a tendency for its perithecia to become clustered and often shows a faint darkened zone about the clusters, but the perithecia remain separately erumpent. In *D. tessella* (Pers.) Rehm, the perithecia are grouped in a slightly differentiated entostromatic area bounded by a definite dark line, but the perithecia are separately erumpent. In *D. sociata* (C. & E.) Sacc., the perithecia are within a strongly differentiated area, bounded by a blackened zone which dips deeply into the wood. The surface of the bark is heavily carbonized between the perithecial necks, forming a blackened "clypeus." In spite of this high development of the stroma, the perithecia have remained separately erumpent.

The majority of the species of the *Diaporthe* group with differentiated entostromatic areas have the clustered perithecia collectively erumpent. The configuration of this entostromatic area may be various. In some species each perithecial group is imbedded in an isolated entostromatic area, as in *D. oxyspora* (Pk.) Sacc., which may or may not be connected with its neighbors by a continuation of this marginal zone. In other cases the entostromatic areas may be more or less effused and contain a number of perithecial clusters, as in *D. impulsiva* (Cke. & Pk.) Sacc., or the perithecia may occasionally be somewhat scattered. The marginal zone may be only dorsal, as in *D. detrusa* (Fr.) Fck., and delimit the upper surface of the area only, or it may also be ventral and dip into the wood, outlining the stromatic area beneath, as in *D. tuberculosa* (Ell.) Sacc. These stromatic characters often vary widely within the same species, as in *D. acerina* (Pk.) Sacc., and can be used only with an understanding of the specific variations.

<sup>4</sup>Given as *Valsa Woolworthii* by Peck (N. Y. State Mus. Rept. 28: 73). Spelling changed to *D. Woolworthii* by Saccardo (Syll. 1: 615).



Ruhland (99) considered the stromatic development of the two Chorostate forms which he studied to be intermediate between his "entoplacodialer" and "ectoplacodialer" types, because the erumpent disc is of ectostromatic tissue but is often obliterated by the growth of the erumpent ostioles. In general the development of ectostromatic tissue is less distinct in the species of the Diaporthe group than in those of the Melanconis group. In the simpler forms the pycnidia are produced in small ectostromatic masses just beneath the periderm, and the scattered perithecia are not oriented in any way in respect to the ectostromata. Where the perithecia are formed in clusters, it is usually beneath these ectostromatic pycnidia. In the higher forms, where an entostromatic mycelium is formed about the perithecia, this ectostromatic disc often remains sterile. In such forms the ectostroma is often not sharply differentiated from the entostroma, and when the pycnidial locules are formed they may be within a stroma which is partially or wholly imbedded within the bark.

For the sake of clearness we shall proceed here to a discussion of the imperfect stages of the Diaporthe group, and discuss the Melanconis group of the genus Diaporthe under the genus Melanconis, in order to bring out the development of what seems to be a separate series of forms.

The imperfect fruit bodies as well as the perithecia of the genus Diaporthe can be derived from the genus Gnomonia. The fact that these imperfect forms have been placed in numerous genera, and even in different orders, of the fungi imperfecti does not disprove their relationship, but rather discloses the artificiality of our classification. Klebahn (67, 69, 72) has given the complete life histories of a number of species of Gnomonia. The imperfect stages of this group consist of thin stromatic patches of mycelium formed within or beneath the cuticle of the host. Upon this basal hymenium is borne the hymenial layer that produces the conidia, which are hyaline, usually one-celled, and elliptical to rod-shaped. In *G. leptostyla* (Fr.) Ces. & de Not. (69), both one-celled, rod-shaped, and 2-celled, crescent-shaped conidia are formed. The probable relation of these 2-celled spores to the septate conidia of certain species of the Melanconis group will be discussed later. The presence of the two types of conidia is common to the genera Diaporthe, Melanconis, and Pseudovalsa.

The classical paper of Klebahn (67) on *Gnomonia veneta* (Sacc. & Speg.) Kleb. (*G. platani* Kleb., or *Apiognomonia veneta* (Sacc. & Speg.) v. H.) points out the tendency toward the formation of a greater amount of stromatic mycelium in the production of the conidial fruit body. On leaves this fungus produces a typical Gloeosporium (*Gl. nervisequum* (Fck.) Sacc.). On dead fallen leaves, a pycnidial stroma more or less imbedded within the leaf tissue is formed, which is known as *Sporonema platani* Baumler, or *Fusicoccum veronense* C. Mass. On twigs the hymenial layer is formed in a cavity beneath a lenticel. On account of the resistance of the overlying cork tissues, this layer is not ruptured widely, and the locule beneath is

lined with the hymenium and the underlying mycelial layer. This fruit body, known as *Discula platani* (Pk.) Sacc., differs from the typical *Phomopsis* of the lower forms of *Diaporthe* only in the slight presence of the outer stromatic wall and in the lack of the two types of spores.

The *Gnomonia* just mentioned is one with unequally 2-celled ascospores, and is placed by von Höhnelt in the genus *Apiognomonia*. *Diaporthe obscura* (Pk.) Sacc., which has been grown in culture by the writer (129), also has unequally 2-celled ascospores and would fall in von Höhnelt's genus *Apioporthes* (57, p. 381). This species has a strongly developed stroma and produces stromatic pycnidia (129, Pl. III, figs. 3, 6), within which are produced conidia that vary in shape from elliptical to cylindric-fusoid and suggest strongly the differentiation into two types of conidia.

The imperfect stages of the species of the *Diaporthe* group are strikingly constant, being, almost universally, species of the genus *Phomopsis*. The species of the *Melanconis* group on the contrary have their conidia borne in open cavities or layers, and one type of conidium is dark-colored, or septate, or both. An examination of the various species of *Diaporthe* connected with the genus *Phomopsis* in Diedicke's (23) article on the latter genus brings out this correlation in a striking manner. Of the 74 specific connections reported by Diedicke, there is only one, *D. leiphaemia*, which belongs to the *Melanconis* group. The connections of this species with its imperfect stage seem to be somewhat confused, but judging from the opinion of Diedicke (23), the figures of the Tulasnes (123, Pl. XXIII, figs. 5-10), and the discussion of von Höhnelt (57), the imperfect fruit body is apparently transitional between *Phomopsis* and *Fusicoccum*. Several of the species mentioned by Diedicke are of the subgenus *Euporthes* and have no dark line; one, *D. veprii* (de Lacr.) Fck., is in reality a *Gnomonia* according to Winter (132, p. 637); and one, *D. nigrella* (Auers.) Niessl., has one-celled spores and belongs to the genus *Diaporthopsis* Fabre (Ann. Sci. Nat. Bot. VI, 15: 35); but all the remaining species are typically of the *Diaporthe* group. The imperfect fruit body of these *Phomopsis* types consists of a spherical, flattened, or somewhat irregular cavity, which is formed within a more or less well developed ectostroma. This ectostroma may occur isolated, or in connection with a perithecial entostroma beneath. There are two types of spores formed, either in separate pycnidia, or usually in the same pycnidium. One type of conidium is long-cylindrical, hyaline, one-celled, and usually curved or hamate; the other type is one-celled, hyaline, elliptical to fusoid, and contains two oil droplets, which von Höhnelt (51, pp. 681, 682) considers an indication of a tendency toward the formation of septate conidia.

The writer has carried five species of this *Diaporthe* group in culture (*D. oncostoma* (Duby) Fck. (127), *D. faginea* (Curr.) Sacc., *D. pruni* E. & E., *D. sp.* and *D. binoculata* (Ell.) Sacc. var. *ilicis* (128)). The first four produced typical *Phomopsis* fruit bodies. *D. binoculata* formed the long-hamate type

of spores in a locule above the perithecial fruit body and the shorter fusoid to cylindrical spores in isolated stromata, which sometimes had more or less exposed cavities.

From the preceding it is seen that in this *Diaporthe* group as here limited we have a correlation between the occurrence of a dark marginal line about the perithecial stroma and the formation of a typical *Phomopsis* fruit body in the conidial stage of the life history.

### *Melanconis*

When one comes to consider that group of species of the genera *Diaporthe*, *Melanconis*, and *Pseudovalsa* without a dark marginal zone, one is impressed with the idea that he is here again dealing with two lines of development.

The stromatic development of these forms is of two types. Many species have a sharply defined, truncate-conical, well developed ectostroma, but practically no entostroma that can be recognized as such; the perithecia are buried in a circinate manner about this central disc in the unaltered bark cortex. A second group of species has no such sharply defined ectostromatic disc. They either show very little stromatic development at all, or, where a stromatic mycelium is developed, it is found in the bark tissues between the perithecial necks, or extending down between the perithecia themselves. There seems, furthermore, to be a correlation between these two types of stroma and the type of imperfect stage produced. The group first mentioned, with a definite ectostroma, has, as a rule, one-celled dark-colored conidia, and represents the genus *Melanconis*. The second group, as far as the evidence goes, has septate, hyaline to colored conidia, and includes the genus *Pseudovalsa*. It must be admitted that the evidence for such a separation is as yet fragmentary and that certain exceptions exist, but let us consider this evidence. Since the old classification cuts across these apparent lines of development, it will be necessary under the discussion of *Melanconis* and *Pseudovalsa* to consider species which have in the past been placed in various other genera. As the possession of dark-colored one-celled conidia has always been considered a character of the genus *Melanconis*, we shall here consider the series of forms possessing this character.

The *Melanconis* group of the genus *Diaporthe* includes such species as *D. marginalis* Pk., *D. albovelata* (B. & C.) Sacc., *D. galericulata* (Tul.) Sacc., *D. Ellisii* Rehm, *D. decipiens* Sacc., and others. The perithecial stroma of these species can be distinguished in no way from the hyaline-spored forms placed in the genus *Melanconis*. The juggling back and forth of many of these species between the two genera shows their position to be mainly one of personal opinion. The presence of dark-colored one-celled conidia (*Melanconium* stage) has been used as a criterion for the separation of these species. Culture experiments of the writer have shown that both *D. marginalis* and *D. albovelata* (127) have dark-colored conidia, and Graves

(38) has transferred the old *Diaporthe juglandis* E. & E. to the genus *Melanconis* on the basis of similar cultural results. It will probably be shown that most of these species of the *Melanconis* group of the genus *Diaporthe* have dark-colored conidia.

Many of these species with a *Melanconium* stage in their life history have a distinctly yellowish or olive-colored ectostroma. The ascospores of this group tend to become colored and lie uniseriately in a cylindrical ascus, especially in the higher forms. The conidial stroma in most cases is that of a typical *Melanconium*. The conidial hymenium, instead of being borne in an enclosed locule as in the *Phomopsis* type belonging to the *Diaporthe* group, is here borne in open cavities. The well developed ectostroma ordinarily bursts through the periderm as a sterile disc. The conidial hymenium is formed in shallow cavities upon the slanting sides and bases of this stroma. In vertical section such a fruit body shows two lateral cavities on either side of the central sterile disc. In some cases in which the growth is rapid, the hymenium develops over the entire surface of the ectostroma before it bursts through the periderm, and as a result it appears in vertical section as a single cavity arched over by the periderm; the undeveloped sterile disc appears as a central dome-shaped projection. If the periderm is widely ruptured the spores are emitted as a mass, while if only a small pore is formed they emerge as a spore horn. This stromatic configuration may vary for one and the same species under different growth conditions. Perithecial stromata may or may not form beneath these conidial ectostromata.

Let us consider a few species which illustrate the development of this group. *D. marginalis* Pk. is a species with hyaline appendiculate ascospores which are biseriate in the ascus. The definite conical ectostromatic disc of this species is white in color. In the writer's cultures (unpublished) it has produced one-celled, elliptical, dilute-blackish conidia, with which were mixed a second type of cylindrical hyaline conidia. The conidial mass is black in color. The conidia are formed on the lateral surface of a central sterile disc, or over the entire surface of a dome-shaped ectostroma.

*Melanconis stilbostoma* (Fr.) Tul. is in many respects similar to the last-mentioned. Its ascospores are hyaline, not appendaged, and sub-biseriate in the ascus. The disc is whitish or yellowish, and the elliptical to ovate conidia are definitely brownish and are accompanied by smaller, rod-shaped, hyaline conidia, according to the figures of the Tulasnes (123, Pl. 14, fig. 4).

*M. chrysostroma* (Fr.) Tul., which is apparently the same as *Diaporthe sulphurea* Fck. and *D. decipiens* Sacc.,<sup>5</sup> has 2-celled hyaline to yellowish ascospores with an obscure apiculus at each end. The spores are biseriate

<sup>5</sup> The exsiccatus of *M. chrysostroma* given out by Ellis (N. A. F. 2d ser., no. 1563) and checked by Winter, is merely an old, over-mature specimen of *D. decipiens* Fck., which Ellis (29, p. 527) says is doubtfully distinct from *D. sulphurea* Fck. Both Winter and Ellis are in doubt as to there being any difference between *D. sulphurea* and *M. chrysostroma*. Petrak (91, p. 292) also remarks upon the similarity of these two species.

in an elongate-clavate ascus. The disc is here distinctly yellowish-green. The Tulasnes figure elliptical to cylindrical, one-celled, hyaline conidia, and also ovate-pyriform brown conidia (123, Pl. XXIV, figs. 14-17). Von Höhnelt (54, p. 198) reports large oval spores in the imperfect stroma (*Discosporium deplanatum* (Lib.) v. H.) of *M. chrysostroma*. These he considers as young conidia of the *Melanconium bicolor*  $\beta$  *ramulorum* Cda. pictured by the Tulasnes. Petrak (91, p. 293) disputes this connection on purely theoretical grounds. These connections are by observation only, and should be checked by culture work.

*Melanconis juglandis* (E. & E.) Graves has hyaline to light-grayish-olive ascospores, which are arranged in an obliquely uniseriate manner in the ascus. This species has a yellowish conical disc. Graves (38) has obtained the oval olive-gray conidia of *Melanconium oblongum* Berk. in his cultures of this fungus. Only spores of this type are found in the pustules in nature. In culture, however, Graves obtained also pycnidia containing small oblong, hyaline conidia.

The spores of *Melanconis Decoraensis* Ell. are 2-celled, brown, and arranged uniseriately in a long-cylindrical ascus. This species has a small yellowish-green disc, and was found by Ellis to be accompanied by a *Melanconium*. The writer has also found a *Melanconium* associated with this species (*Mycologia* 18: 263).

*Melanconis spodiaea* Tul., which according to Ellis differs from the preceding species only in its appendaged spores, is described by Winter as having a yellow-green disc and brown ascospores, arranged uniseriately to biseriately in a cylindrical ascus. The Tulasnes (123, Pl. XXIV, figs. 12, 13) figure both ovate brown and cylindrical hyaline conidia for this species also.

Two species should be mentioned here which are apparently exceptions to the general structure of this group. Both of these species, *Diaporthe galericulata* (Tul.) Sacc. and *D. albovelata* (B. & C.) Sacc., lack any marginal zone but show a well developed entostroma and a poorly defined ectostroma. *D. galericulata* was studied in culture by the writer (129), but produced no conidial fruit bodies. A Fusicoccum with hyaline conidia is reported as belonging to this species. This connection is only by association, and may prove to be incorrect. *D. albovelata*, in the writer's (127) cultures on sterile twigs of *Tilia americana* L., formed a sporodochia type of imperfect stage (*Sporocybe Rhois* (B. & C.) Sacc.) with one-celled colored spores. There were formed, however, on both agar and twig cultures, all transitional forms between the long-cylindrical fruit bodies of *Sporocybe* and loosely stromatic structures of a pulvinate shape, within which similar spores were formed. Pycnidial cavities containing one-celled, hyaline, fusoid-elliptical conidia were also found above the perithecial stromata. This may be considered as a species with a specially developed imperfect stage.

The preceding species, although they have been placed arbitrarily in

either the genus *Diaporthe* or the genus *Melanconis*, show a close relationship in the presence of a well defined, often colored, ectostromatic disc; in the absence of any recognizable entostroma; and in the possession of a *Melanconium* stage in their life history. The two species *D. galericulata* and *D. albovelata* are somewhat doubtful in their relationships and must await further study of other species of this group for a determination of their true position. In addition to these related forms, there remain certain species that have been placed in the genus *Melanconis* whose relationships seem to lie with the genus *Pseudovalsa* and which will be discussed under that genus.

#### *Pseudovalsa*

The genus *Pseudovalsa* is usually distinguished from *Melanconis* by its many-celled ascospores and by the possession of a *Coryneum* or *Stilbospora* stage in the life history. In the following discussion certain species of *Diaporthe* and *Melanconis* with a similar stromatic development and septate conidia will also be considered. Cultural life histories are badly needed to supplement the incomplete, and in many cases unreliable, knowledge of the imperfect stages of this group. The conidial stages of these species so far reported seem to point to a very interesting development of the forms now placed in *Pseudovalsa*.

The imperfect stages of this group have one type of conidium which is either hyaline or colored and septate, and usually a second type in the same species which is short, rod-shaped, more or less curved, hyaline, and one-celled. The hymenial cavities of the conidial stromata are more variable in these than in the *Melanconium* forms. These cavities may be either on the flanks of a central sterile disc, more or less sunken in the surface of the dome-shaped surface of the ectostroma, or may be entirely enclosed so as to form locules within the stroma.

We have previously noted the occurrence of both two-celled, hyaline, crescent-shaped, and one-celled, hyaline, rod-shaped conidia in *Gnomonia leptostyla* (Fr.) Ces. & de Not. Although the evidence as to the origin of these forms with septate conidia is unsatisfactory and fragmentary, this species presents an interesting possibility as an ancestral form of the group.

For the first examples of species of this type in the genus *Diaporthe*, let us examine the segregated genus *Cryptodiaporthe* of Petrak (89, p. 117). Petrak erected this genus for certain species of *Diaporthe* which show little or no development of entostromatic mycelium about the perithecia, which in turn are rather loosely aggregated. In this group he has included *D. aesculi* (Fck.) v. H., *D. Hystrix* (Fck.) Sacc., *D. spina* Fck., and *D. salicella* Sacc. Petrak found *Septomyxa aesculi* Sacc., with 2-celled conidia, associated with *D. aesculi*, and therefore supposes that this group is characterized by a *Septomyxa* stage. He transfers *Discella carbonacea* (Fr.) Berk. & Br., which he previously published as the imperfect stage of *Diaporthe tessella*, to *D. salicella* upon this supposition. The species of *Septomyxa*, he points

out correctly, are merely variations of *Discella* forms with more open hymenial cavities. These connections, to be sure, are by association only and some of them smack strongly of the personal equation, but they are very suggestive nevertheless, and if such connections can be substantiated by cultural studies they will show the origin of septate conidia among the lower forms.

*Diaporthe furfuracea* (Fr.) Sacc., which was carried in culture by the writer (127), is interesting in this connection. In culture, this species produced long, fusoid-cylindrical, hyaline conidia with 1-3 very faint septa. These septa are nearly obscured by the numerous oil drops present, but can be brought out by treating the spores with a solution of phenol in xylol. In agar the conidia were abnormally bent or twisted, but showed a definite central septum. These spores were whitish in mass. A second type<sup>6</sup> of short, hyaline, one-celled, rod-shaped conidia was also formed, which were greenish-black in mass. In many cases the conidia were formed in enclosed locules beneath the surface hymenial cavities, as well as in these surface layers. This species does not agree entirely with the above-described group, since there is often present a thin but distinct darkened zone about the perithecial clusters. We shall see as we proceed, however, that there are other species of both *Melanconis* and *Pseudovalsa* which show a faint darkened zone. This species was carried in culture for two years before conidia of the rod-shaped type were produced, and then only sparsely on the terminal portions of sterile twigs. This is interesting evidence for the view that all the species of *Diaporthe*, *Melanconis*, and *Pseudovalsa* possess the potentiality of producing two types of conidia. Where one of these types is not known, we may consider that it is merely suppressed and would appear under the proper conditions.

Certain species which have been placed in the genus *Melanconis*, but which lack a definite ectostromatic disc, provide us with the next step in the development of this series.

*Melanconis occulta* (Fck.) Sacc.<sup>7</sup> has perithecia in clusters of 2-4, and a disc composed of the fused and enlarged perithecial necks. The ascospores are large ( $38-45 \times 13-15 \mu$ ), and have a broad, hyaline, recurved appendage at each end. The asci are broadly clavate, and the spores are biserially arranged. There seems to be no authentic connection of an imperfect form. *M. Everhartii* Ell. is very similar to the last-named species, having a disc composed merely of the thickened perithecial necks. The ascospores are

<sup>6</sup> Both types of these conidia have since been found in nature on *Tilia americana*, in association with *D. furfuracea*.

<sup>7</sup> This species has been placed in the genus *Diaporthe* by von Höhnelt (57, p. 387) on the basis of the absence of paraphyses. Petrak has reported the absence of paraphyses from *M. spodiacea* and *M. appendiculata*, and erected the sub-family "Pseudodiaporthen" for the dark-spored species of *Melanconis* (*Melanconiella*) without paraphyses. The presence and absence of paraphyses has been proved or disproved for nearly every species of *Melanconis*, and this is a very unreliable character, especially when determinations are made from dried herbarium material.

large ( $25-38 \times 8-11 \mu$ ), have a broad, curved appendage at each end, and are arranged biserially in a broad, clavate ascus. The writer knows of no conidial connection for this species.

In the case of *M. thelebola* (Fr.) Sacc., we have an interesting transition. In this species, as in *Diaporthe furfuracea*, we often find a faint, dark, marginal line. The ascospores are large, hyaline, usually 2-celled, and with a long setaceous appendage at each end. The writer has seen these ascospores with 2-3 septa when fully mature. There is often a development of stromatic mycelium about the perithecial necks, but this is chiefly entostromatic. The Tulasnes (123, Pl. XXI, figs. 3-12) figure enclosed locules within this stroma, in which are formed two types of conidia.<sup>8</sup> One type is a short, curved, rod-shaped, one-celled, hyaline conidium, the other is larger, clavate, brown, and 3-septate. We have here a species with 2-celled hyaline ascospores (which may become 2- to 3-septate), which shows the typical brown, pluriseptate conidia of the genus *Pseudovalsa*. The formation of these conidia within a locule, which occurs in this species, is a variation that, as we have seen, is common in the lower forms of this group.

In *Melanconis modonia* Tul. the bark tissues above and about the perithecia are rather heavily blackened and interspersed with entostromatic mycelium. The ascospores are large ( $27-35 \times 10-11 \mu$ ), irregularly biserial in the ascus, and according to the descriptions become light brown at maturity. Petrak (94, p. 323) recognizes this species as transitional between *Melanconis* and *Pseudovalsa*. He reports that the ascospores become 2- to 3-septate at maturity, and upon this fact and the character of the stroma places it in *Pseudovalsa*. This species has a *Coryneum* with 3- to 8-septate brown spores for its imperfect stage. This imperfect stage with the conidia borne on the surface of the stroma is characteristic of the genus *Pseudovalsa*. The Tulasnes (123, Pl. XV, figs. 1-5) show this spore form and also a second, consisting of small, rod-shaped to allantoid, hyaline, one-celled conidia, formed in open cavities on the sides of a central sterile disc.

The preceding species of *Diaporthe* and *Melanconis* are similar in the presence of septate conidia borne in shallow cavities or in more or less enclosed locules within a stroma. There is a general lack of a sharply defined ectostroma. We can note a general tendency towards the septation and coloration of both the ascospores and the conidia of one type. The ascospores become large, but remain biserial in the ascus; they also become appendaged in many species, but this is not a distinctive character, since it also occurs in the other two groups.

The species of *Pseudovalsa* show a continuation of the development traced in the preceding species. The ascospores of the genus *Calosporella*,

<sup>8</sup> Von Höhnelt has erected the genus *Pseudovalsella* for this species on account of the conidial connections. He designates the pycnidia with rod-shaped conidia as *Cytosporopsis umbrinus* (Bon.) v. H., and the pycnidia with the brown septate conidia as *Hendersoniopsis thelebola* (Sacc.) v. H., in spite of the fact that the Tulasnes picture both types of conidia in the same locule.



which are 4-celled and hyaline, have been variously interpreted as 4-celled Diaporthe spores or hyaline Pseudovalsa spores, which fact shows that they are merely transitional species. The spores of many species of Pseudovalsa remain hyaline until almost mature. Petrak (94, p. 323) points out that *P. aucta* (Berk. & Br.) Sacc. is a transitional species between *Melanconis* and *Pseudovalsa*, since the ascospores remain 2-celled and hyaline for some time.

Petrak (94, p. 323) divides the genus *Pseudovalsa* into two genera. He places in the genus *Prosthecium* Fres. those species which develop little or no entostromatic mycelium, have few paraphyses, and possess appendiculate ascospores. These species, it can be seen from these characters, are closely related to the forms of *Melanconis* discussed under this genus. In the genus *Pseudovalsa*, Petrak retains those species with a well developed entostroma in which the perithecia are usually developed. The ascospores in these forms do not possess appendages. These species have a faint darkened marginal zone, as we have noted for *Diaporthe furfuracea*, *Melanconis modonia*, and *M. thelebola*, and may be considered the climax type of this series.

Many species of *Pseudovalsa*, as *P. lanciformis* (Fr.) Ces. & de Not., *P. umbonata* (Tul.) Sacc., *P. longipes* (Tul.) Sacc., *P. Berkeleyi* Sacc., *P. stilbospora* Auers., and *P. sigmoidea* (Cke. & Ell.) Sacc., have been reported as having either a *Coryneum* or a *Stilbospora* type of imperfect stage; which means that the elongated, many-celled, brown conidia are borne upon the surface of an ectostromatic cushion, which may or may not be erumpent through the periderm. This represents the ultimate development of the more or less chambered fruit bodies with hyaline or brown, septate conidia, found in the forms previously discussed. A second type of short, rod-shaped, more or less curved, hyaline, one-celled conidium is also reported for some species of this genus. Such conidia are figured by the Tulasnes for *P. umbonata* (123, Pl. XV, fig. 9) and *P. lanciformis* (Pl. XVI, figs. 9-11). Von Höhnelt (51, p. 683) reports that a *Cytospora* (*Dothiorella*?) belongs to *P. Berkeleyi*.

The writer (*Mycologia* 18:264) has had only one *Pseudovalsa* (*P. longipes* (Tul.) Sacc.) in culture. This species produced a *Coryneum* stage on *Tilia*, and, although it produced the second type of filiform hyaline conidia, this occurred only on sterile twigs of *Quercus*. These conidia are borne on the irregular exposed surface of a black, erumpent ectostroma, composed of erect septate hyphae. This species again illustrates the formation of the two types of conidia under different environmental conditions; in this case it was a difference of host substrata.<sup>9</sup>

#### Summary (First Series)

In conclusion let us summarize the main phylogenetic outlines of this series. In the first place, we have traced the origin of the series from

<sup>9</sup> Since this was written the filiform type of conidium has been obtained upon twigs of *Tilia* which were coated with paraffin before being inoculated.

species similar to those of the genus *Gnomonia*. The genus *Diaporthe*, as it has been limited in this discussion, consists of a compact group in which the formation of an entostroma has taken place early in the evolutionary development of the genus. The strong development of a hyaline entostroma has produced in these species a light-colored, differentiated entostromatic area, circumscribed by a dark marginal zone. The imperfect stage of these forms consists of a *Phomopsis* type of fruit body, with an enclosed locule formed in an ectostroma which is intimately associated with the entostroma beneath, when that tissue is well developed. The conidia are of two types, both one-celled and hyaline. The ascospores in this group remain hyaline, 2-celled, and biseriate in the ascus, but may become appendaged in some forms. The paraphyses are delicate, and very few remain in the mature perithecium.

Developing parallel to this *Diaporthe* group we find a second series which does not produce a dark marginal zone, at least until late in the history of its development. This series in turn shows two sub-divisions.

In the first of these sub-divisions (*Melanconis*) the stromatic development is limited to an ectostromatic disc, and the perithecia are imbedded in the unaltered cortex. The ascospores of this series, which culminates in the genus *Melanconis* (*Melanconiella*), become colored and uniseriate in the ascus in the higher forms, but remain 2-celled. The paraphyses become more numerous in this group than in *Diaporthe*, but are still evanescent. The imperfect stage is typically a *Melanconium*, with the conidia borne in shallow cavities on the sides of a sterile disc, or over the entire surface of a dome-shaped ectostroma. The conidia are of two types. One type is elliptical to ovate, one-celled, and more or less dark-colored; the second type is smaller, oblong to rod-shaped, one-celled, and hyaline. In some of the higher forms of the group, the second type is suppressed and occurs only rarely.

In the second sub-division of this second group there is no definite ectostroma, and, where a stromatic mycelium is formed, it is within the bark about the perithecial necks and the perithecia. The origin of this group is doubtful, and further cultural studies of the lower forms are needed. Where a development of an entostroma takes place a faint dark marginal zone appears, but the entostromatic mycelium is usually dark-colored and the stromatic areas are not light-colored, as in *Diaporthe*. The ascospores become dark-colored, and in the higher forms 2- to many-septate. The ascospores of a number of species are appendiculate. The paraphyses show a higher development in this series, and become very numerous in some of the higher forms.

The imperfect stage of this *Pseudovalsa* group is ectostromatic, but these ectostromata are usually not connected with the perithecial stroma, or where they are, as in some species of *Pseudovalsa*, they are reduced. The imperfect fruit body consists of either shallow cavities on the sides of a

sterile disc, a more or less open layer over the entire dome-shaped surface of the ectostroma, or enclosed locules within the ectostroma. Two types of conidia are formed. One type consists of elongate-elliptical to clavate, one- to many-septate, hyaline or brown spores. The second type consists of smaller, rod-shaped, curved, one-celled, hyaline conidia, which are borne in the same cavity as the first type or in separate cavities or even in separate stromata.

If space permitted, the writer would like to discuss in detail the confusion that has arisen as a result of the fragmentary considerations of the three genera just discussed. The separation of these genera has been necessarily vague, since they are integral parts of the same developmental series. They have been separated on groups of characters, such as spore-color and septation, presence or absence of paraphyses, and type of imperfect fruit body, all of which vary independently.

Time and again, the European mycologists working upon this group have transferred species or erected new genera on a consideration of only one or another of these varying characters, or by a consideration of only one or a few species, without any regard to the general relationships within the group. As a result, we have a large number of genera, as *Diaporthe*, *Melanconis*, *Melanconiella*, *Cryptodiaporthe*, *Allantoporthes*, *Apioporthes*, *Discodiaporthe*, *Calospora*, *Calosporella*, *Pseudovalsella*, *Prosthemia*, *Pseudovalsa*, *Aglaospora*, and many others, whose limits are vague and overlapping, and all of which have been delimited from closely related groups of species. If these workers would not insist on creating and tearing down genera on fragmentary considerations of one or a few species, and on frequently incorrect observational inferences concerning the connection of the imperfect stage, but would instead stop to determine definitely by cultural methods the questions presented, we should advance more rapidly to an understanding of the true relationships existing between genera instead of remaining constantly entangled in the confusion of contradictory synonymies.

From the implications that might be derived from the preceding facts, it may be suggested, in a tentative way, that the only genera with sufficiently valid background are those which represent a developmental series, or an integral portion of such a series, such as the three groups we have just outlined.

#### *Second Series*

Under this second series we shall consider certain genera with one-celled ascospores. The main line of development in this group (*Cryptosporella* and *Cryptospora*) shows a similar stromatic structure and a similar imperfect stage. The relationships among the lower forms of the series are still rather vague. The genera *Gnomoniella*, *Mamianiella*, *Diaporthopsis*, and *Mazzantia* all probably represent small groups with a special development of the stroma. *Gnomoniella* has no stromatic development, and may be

considered as the ancestral form. *Sphaerognomonia* shows the development of a stromatic "clypeus" about the ostiole, while *Mamianiella* shows a well developed stroma about the perithecium. These three genera are leaf-inhabiting. The genus *Mazzantia*, as von Höhnelt (60, p. 109) points out, has a stromatic structure identical with that of a well developed *Diaporthe*, but has one-celled ascospores. *M. galii* (Fr.) Mont. shows a strongly developed, differentiated entostroma within the tissue of the substratum, which is bounded by a darkened zone. Von Höhnelt has erected the form genus *Mazzantiella* for the imperfect forms of *Mazzantia*. In these fruit bodies an enclosed locule is formed within the stroma. The conidia are cylindrical and hyaline.

The writer has not examined any species of the genus *Diaporthopsis*, but, judging from descriptions, they form a more or less effused entostroma which is often bounded by a faintly darkened zone. Diedicke gives a *Phomopsis* as the imperfect stage of *Diaporthe* (*Diaporthopsis*) *nigrella* (Auers.) Niessl. Von Höhnelt (60, p. 114) considers this genus as closely related to the subgenus *Euporthe*, or more likely to the *Hyponectriaceae*.

Whether or not some species of *Diaporthe* may have arisen from such forms with one-celled ascospores and rather well developed stromata, is a question which can not be answered at present. It is true that some species of *Diaporthe* show ascospores which are tardily or very faintly septate. Some such species have been described under the genus *Cryptosporella*. On the other hand, there are numerous species of *Diaporthe* with very simple stromatic structure which nevertheless show definitely septate ascospores.

#### *Cryptosporella and Cryptospora*

*Cryptosporella* and *Cryptospora* are the characteristic genera of this series. Von Höhnelt (60, p. 106) noted the "euvalsoid" character of the perithecial centrum in these two genera, and placed them in his "*Diaporthen*."

The species of both *Cryptosporella* and *Cryptospora* are characterized by a sharply defined conical ectostromatic disc, about which the perithecia are arranged circinately within the unaltered bark cortex. There is no differentiated entostromatic area nor dark marginal zone. The imperfect stages of these two genera have been placed in the form genera *Fusicoccum* and *Disculina* (*Cryptosporium*). The structure of these fruit bodies is very similar to that of those found in the first series. They consist of an ectostromatic cushion which contains open cavities or enclosed locules, usually on the marginal portions of the ectostroma. The conidia are one-celled, hyaline, and usually more or less elongated.

The characters just mentioned show a relationship between these two genera and those species which were discussed under the genus *Melanconis*. The chief differences are the non-septate ascospores and the hyaline, usually elongated conidia. In some species of *Cryptospora* (Sillia) the ascospores

become septate, but the long-cylindrical shape easily distinguishes these spores.

According to von Höhnelt (60, p. 106), *Cryptosporella populina* (Fck.) Sacc. has a faint septum in its ascospores, and is the same as *Diaporthe populea* Sacc. Von Höhnelt gives a Phomopsis as the imperfect stage of this fungus. He also gives a Phomopsis as the imperfect stage of *C. Niesslii* (Kze.) Sacc., which has one-celled ascospores and which he considers as synonymous with *Diaporthe hystrix* (Tode) Sacc. An examination of this species (Syd. Myc. Germ. no. 2135) by the writer has shown that it has a stromatic structure typical of *Cryptosporella*, with a well developed ectostroma surrounded by the circinate perithecia in the unaltered bark cortex. Associated with these stromata, furthermore, was found an imperfect stage consisting of ectostromatic cushions with shallow, more or less exposed cavities on their sloping sides. These cavities contained one-celled, hyaline, elliptical to cylindrical conidia  $6.5-13 \times 2.5 \mu$ . These cavities may sometimes be formed more deeply within the stroma, and then resemble a Phomopsis, but the structure is typically that of a *Cryptosporella*. As we have previously seen, Petrak has placed both *Diaporthe hystrix* and *D. populea* in his genus *Cryptodiaporthe*, which is supposed to have *Septomyxa* as its conidial stage. All of which merely shows the necessity for cultural connections and for a clearer conception of the form genera concerned.

Other species have been placed here, as for example *Cryptospora pennsylvanica* (B. & C.) Ell., *C. aculeans* (Schw.) Ell., and *Cryptosporella anomala* (Pk.) Sacc., whose relationships are not with this group, but the important point is that the true *Cryptosporellas* show a correlation of characters. They have one-celled, elliptical to fusoid ascospores, the stromatic characters previously described, and an imperfect stage consisting of one-celled, hyaline, elliptical to fusoid conidia formed in more or less open cavities or locules in the surface of an ectostroma. Von Höhnelt (60, p. 106) gives the imperfect stage of *C. Daldiniana* as *Fusicoccum Lesourdianum* Sacc. & R., and that of *C. aurea* as *F. amygdalinum* (Sacc.) v. H.

The genus *Cryptospora* is similar in all respects to *Cryptosporella*, except that the ascospores are long-cylindrical instead of elliptical or fusoid. The stromatic characters are the same. The imperfect stage is very similar, except that the conidial hymenium is usually more exposed, and the conidia are long-cylindrical and slightly curved instead of fusoid-elliptical. This type of fruit body falls in the form genus *Cryptosporium* Sacc. or *Disculina* v. H. The Tulasnes (123, Pl. XVII, figs. 13, 15, 28-30) figure such imperfect stages for *C. suffusa* (Fr.) Tul. (*Disculina Neesii* (Cda.) v. H.), and for *C. betulae* Tul. (*Disculina betulina* (Sacc.) v. H.). The Tulasnes (123, p. 175) reported spermagonia with hyaline allantoid conidia  $13 \times 2.5 \mu$  associated with *C. corylina*. Von Höhnelt (60, p. 108) reports a *Disculina* (*D. corylina* v. H.) associated with this species also. According to Allescher (2, p. 745), Fuckel reports similar small, cylindrical, hyaline conidia in the hymenial

layer of *Fusicoccum amygdalinum*, the imperfect stage of *Cryptosporella aurea*.

The writer has had two species of *Cryptospora* in culture (unpublished). *C. (Sillia) cinctula* (Cke. & Pk.) Sacc. failed to fruit either on agar or on sterile twigs of *Castanea*. Cultures from a specimen of *C. suffusa* (Fr.) Tul. on *Hamamelis virginiana* L. formed a conidial stage on sterile twigs of *Hamamelis*. A pulvinate ectostroma was formed, composed of parallel septate, greenish-black hyphae. On the lateral portions of this ectostroma locules were formed, which soon opened to the exterior, forming shallow cavities. The conidia produced in these cavities were oblong-cylindrical, one-celled, hyaline, and  $9-13 \times 2.5 \mu$ . This formation of small cylindrical conidia in culture, together with the two cases already mentioned in which such conidia were found in connection with *Cryptospora* and *Cryptosporella*, shows that the species of this series may also produce two types of conidia, and completes the resemblance of this series to the one previously discussed.

Von Höhnelt (60, p. 108) has placed certain species of *Cryptospora* which show one or more cross walls in their ascospores in the genus *Sillia*. The writer knows of no conidial connection with any of these species.

In conclusion, we see that this second series of the *Diaporthaceae* differs from the first chiefly in the non-septate character of the ascospores (becoming faintly septate in some species of *Cryptospora*), and in the non-septate, hyaline character of the conidia. The presence of a sharply defined ectostroma, the lack of either an entostroma or a darkened zone, and the non-septate conidia place them in closest relation with the species previously discussed under the genus *Melanconis*.

The lower forms, as we have seen, present several small specialized groups with more or less well developed stromata. *Cryptosporella* and *Cryptospora* show a short line of development, with a tendency towards the elongation and septation of the ascospores and a similar elongation of the conidia. *Mazzantia*, *Cryptospora*, and *Cryptosporella* all seem to be valid genera of this series.

### *Third Series*

The third series of related forms within the *Diaporthaceae* includes the genera *Valsa*, *Leucostoma*, *Valsella*, *Endothia*, and *Valsaria*. *Leucostoma* will here be considered as a separate genus on account of its distinctive stromatic development. Von Höhnelt (60, p. 130) separates *Leucostoma* as a genus on the basis of the white disc, the long perithecial necks, and the well developed entostroma. Petrak (89, p. 128) rightly points out that the first two characters do not distinguish *Leucostoma* from *Euvalsa*. He discards the stromatic character as a differentiating one because, as he says, certain species of *Euvalsa* have this character. This stromatic development is, however, the differential character, and if these species (which he does not mention) possess such stromatic characters they belong in *Leucostoma* and not in *Euvalsa*.

Von Höhnelt (60, p. 130) places *Valsa*, *Leucostoma*, and *Valsella* in the "Valseen," a separate sub-family of his "Diaportheen," in contrast to his "Eu-Diaportheen" in which he includes all the genera discussed in the first two series. This seems to the writer a logical separation on the basis of general relationships. We have seen in the previous discussion that both the perfect and the imperfect stages of the first two series are very similar in structure, the difference between the two series being mainly one of spore characters.

In this third series, however, we find quite marked differences in both stages of the life history from the species of the first two series. In *Euvalsa* the structure is quite simple and resembles that of *Melanconis*, but in the other genera there is a well developed and strongly differentiated entostroma. The ascospores of all the species of this series, except some species of *Endothia* and *Valsaria*, are, moreover, allantoid, hyaline, and one-celled. The imperfect stage also is here quite different. The conidial chambers in this series are numerous or labyrinthiform, and are produced not only in the ectostromatic but also in the entostromatic tissues. The conidia are small, one-celled, hyaline, and cylindrical to allantoid.

The genus *Glomerella*, with its allantoid ascospores, presents a possible origin for this series among the simple *Sphaeriales*. There seems to be, however, a rather wide gap between such forms and the species of *Valsa*.

### *Valsa*

*Valsa* is a large, but distinct and compact genus. In the older classifications it was usually placed with the genera here placed in the *Allantosphæriaceae*. It differs from these genera in the character of its perithecial centrum, its hyaline ascospores, non-stipitate asci, non-sulcate ostioles, the lack of any dark marginal zone, and in the character of its imperfect stage; there remains only a very superficial resemblance.

The species of this genus have a definitely differentiated truncate-conical ectostroma, which forms the erumpent disc. The perithecia are buried in the bark cortex beneath this ectostroma. There may be a slight development of entostromatic mycelium about the perithecia, but there is no differentiated area, and even under a hand-lens the perithecia appear to be imbedded in the unaltered bark cortex. Ruhland (99), in his discussion of *Valsa salicina* (Pers.) Fr., contradicts himself in regard to the character of the ectostroma. On page 52 he says: "Wie überhaupt bei den Arten der Subgenera *Leucostoma* und *Euvalsa* ist auch hier die Grenze zwischen Ecto- und Entostroma auf ausgebildeten Stadien keine scharfe." Later, on page 54, he says for the same species: "Das Ectostroma, welches stets gut von dem mycelartigen Entostroma abgesetzt ist . . . ." The entostroma of this species is visible only under the microscope, while the ectostroma is very sharply defined.

The imperfect stage of the genus *Valsa* is very constant, belonging to

the form genus *Cytospora*. The fruit bodies consist of a stromatic tissue in which there are formed numerous locules, which often coalesce to form a labyrinthiform chamber. The conidia are small, allantoid, one-celled, hyaline, and ejected in large numbers in the form of a spore horn. In both *Valsa* and *Leucostoma* the pycnidial locules arise in the same tissue as the perithecia, but the two types of fruit bodies are practically never found in the same stroma. The pycnidial locules of the species of the genus *Valsa* arise in mats of entostromatic mycelium which are formed within the bark tissues. These entostromatic masses of mycelium are not very extensive, and there is comparatively little stromatic tissue remaining about the locules at maturity. The result is a thin-walled labyrinthiform pycnidium imbedded in the cortex, which is characteristic of this genus.

### *Leucostoma*

The stromatic development of the species of the genus *Leucostoma* is quite different from that of species of the genus *Valsa*. As Ruhland (99) has pointed out in his discussion of *V. (Leucostoma) nivea* (Pers.) Fr. and *V. (Leucostoma) superficialis* Nit., the differentiation between ectostroma and entostroma is very vague in this genus. In some species, as *V. (Leucostoma) subclypeata* Cke. & Pk., there is formed a differentiated cap of ectostromatic mycelium, but in many species this is very poorly developed and scarcely distinguishable. In all the species of this genus the stromatic area is delimited very early in its development by a dark marginal zone of tissue, which limits the size of the stroma. The position of this delimiting zone varies in the different species. In *V. (Leucostoma) leucostoma* (Pers.) Fr. this zone runs just beneath the periderm, and the stroma develops between the periderm and the bark surface. In other cases, as *V. (Leucostoma) nivea*, *V. (Leucostoma) translucens* (de Not.) Ces. & de Not., and *V. (Leucostoma) superficialis*, this zone may penetrate into the bark to a depth of 3 to 5 layers of cells. The perithecia may then develop either in or on the surface layers of the bark. In *V. (Leucostoma) subclypeata* this blackened zone penetrates deeply into the bark, cutting off a spherical area within which the bark cells are absorbed and replaced by a stromatic fungous tissue. In all the preceding cases there is a strong development of stromatic mycelium about the perithecia, so that the mature stroma is composed almost entirely of fungous tissue, with only a few disintegrated remains of the bark cells. It can be seen that the terms ecto- and entostroma, as defined at the beginning of this discussion, apparently break down here. Where there is a differentiated cap of tissue formed which functions in the opening of the periderm, the stromatic tissue in which the perithecia are imbedded should undoubtedly be considered as entostroma. In many cases, however, this tissue develops entirely upon the bark surface. Where it does arise in the surface bark layers, these are soon absorbed or imbedded in the fungous tissue. Even where the darkened zone penetrates into the



layers of bark cells, these cells often remain comparatively intact and the perithecial stroma develops above them. Ruhland (99) considers this tissue in which the perithecia are imbedded as entostroma, and this is probably the correct interpretation, since many species show a differentiated ectostroma above it.

The pycnidial locules of this genus, which are numerous or labyrinthiform, are produced in a stroma identical with that in which the perithecia are formed, except that in some cases the tissues of the pycnidial stroma become blackened and so obscure the bounding zone. The pycnidia of the species of *Leucostoma* show a greater stromatic development than those of the genus *Valsa*, corresponding to such a development in the perithecial stromata. Von Höhnelt (60, p. 130) has erected the form genus *Leuco-cytospora* for this more stromatic type of *Cytospora* fruit body.

#### *Valsella*

The genus *Valsella* differs from *Leucostoma* only in its polysporous asci. As has previously been noted under the discussion of the polysporous *Allantosphaeriaceae*, Petrak (94, p. 227) considers the species of *Valsella* as not distinct from certain corresponding species of *Leucostoma*, but merely polysporous forms of these produced under certain conditions. Although Sydow's Myc. Germ. no. 2132 of *V. (Leucostoma) translucens*, distributed as an example of the association of such 8-spored and polyspored forms, seems to bear out this view, its validity can be determined finally only by cultural proof. The writer (128) has obtained perithecia of *V. (Leucostoma) Kunzei* Fr. in culture, but has not as yet observed the occurrence of any polysporous asci.

The imperfect stage of the genus *Valsella* is the same as that found in *Leucostoma*.

#### *Endothia*

Von Höhnelt places the genus *Endothia* in his "Diaportheen" on the basis of the structure of the perithecial centrum. This genus was excluded from his "Valseen," apparently because that subfamily includes only allantoid-spored forms. Both *Endothia gyrosa* (Schw.) Fr. and *E. singularis* (H. & P. Sydow) S. & S., however, have long-elliptical to allantoid, one-celled or pseudo-septate ascospores, and this fact, together with the formation of stromatic pycnidia containing labyrinthiform locules, suggests to the writer that the position of this genus is close to that of *Leucostoma*.

The development of the stroma in *Endothia* is variable and often difficult of interpretation. In *E. parasitica* (Murr.) P. J. & H. W. And., as pointed out by P. J. Anderson (3), the stroma usually originates as a hyaline ectostromatic cushion just beneath the periderm. The amount of this tissue formed varies widely. If a sufficiently well developed ectostroma is produced, pycnidial locules are formed within it. If this tissue is much reduced it remains sterile, and unless pycnidial locules are formed within the

entostroma, which may also occur, there results a stroma with perithecia only. About the time the ectostroma ruptures the periderm its tissues become tinted a yellow, and finally a reddish-orange color. The perithecial initials are formed within the bark cortex beneath the ectostroma. Along with the development of the perithecia there is a formation of entostromatic mycelium. This entostromatic development is often very vigorous, and a large stromatic mass containing only the remnants of the bark cells is pushed up through the periderm. Wherever the stroma becomes exposed in this manner it undergoes the characteristic coloration. In these larger stromata the ectostroma can no longer be distinguished. In a less well developed stroma it can often be seen, however, as a conical orange-colored disc. Shear's (110, p. 26) statement that the stroma of *E. parasitica* can not be divided into ecto- and entostroma seems to be based upon the supposition that these terms are synonymous with conidio- and ascostroma. Many of his figures show the differentiation of two types of tissue. In his Plate XV, figure 2, he shows two sterile ectostromata; figure 1 shows an ectostroma with a pycnidial locule forming in its base; and figure 3 shows pycnidial locules forming within the entostroma. Plate XIV, figure 2 of the same bulletin shows a sterile ectostroma with both a perithecium and a pycnidial locule forming in the entostroma beneath, while Plate X, figure 1 shows a slightly developed ectostroma with the perithecia beneath in the entostroma.

Such species as *E. gyrosa* and *E. singularis* show an exceptional growth of entostroma, resulting in a large crumbly mass of fungous tissue in which only the remnants of the bark cells can be found. This type of entostromatic development is similar to that already noted in the genus *Valsaria*.

In this genus the entostromatic development is not limited by the formation of a blackened limiting zone, as in *Leucostoma*, and the stromata become large and irregular in shape. The coloration of the stromatic tissues, the septation of the spores, and the cylindrical rather than allantoid conidia also separate it from *Valsa* and *Leucostoma*. The forms of this genus must have arisen therefore as a divergent line from some common ancestor of these other two genera.

### *Valsaria*

The relationships of this genus and the reasons for placing certain species in the *Allantosphaeriaceae* have been discussed under that series. It remains here to show the similarities between such species as *V. insitwa* Ces. & de Not. and *V. exasperans* (Ger.) E. & E. and the preceding genera of this series.

These species of *Valsaria* differ from *Endothia* in the uniseriate, colored ascospores, the numerous paraphyses, and the total lack of any ectostromatic tissue. The structure of the ascus is, however, typical of the *Diaporthaceae*. The imperfect stage with its elliptical conidia formed within labyrinthiform locules is almost identical with that of *Endothia*, except that no ectostromatic

locules are formed. The coloration of the stroma, although black in *V. insitiva*, brown or mouse-gray in *V. exasperans*, and dull red in *V. rubricosa*, is also suggestive of the genus *Endothia*. The dark zone outlining the stroma, particularly in *V. exasperans*, is similar to that found in *Leucostoma*.

The preceding facts point to a common ancestral form for the genera *Leucostoma*, *Endothia*, and *Valsaria*. The dark-brown spores and the lack of any transitional forms, however, make the position of the genus *Valsaria* in this series only tentative.

This third series of the *Diaporthaceae* stands out in contrast to the other two series by virtue of its quite different imperfect stage. The genus *Valsa* presents the simplest forms. The genus *Leucostoma* differs only in the strongly developed entostromatic mycelium, which arises about the perithecia within a limiting blackened zone. The species of *Endothia*, arising probably from similar simple forms of a *Valsoid* type, have a different type of stromatic development and have developed a septate, elliptical ascospore. The genus *Valsaria* stands in a somewhat intermediate position, with the stromatic development of *Leucostoma* but with ascospores similar to *Endothia* but brown in color.

#### *Summary (Diaporthaceae)*

In the *Diaporthaceae*, as in the *Allantosphaeriaceae*, we have seen the development of the stroma taking place in several separate series of forms. These separate developmental series can be distinguished in this family by a constancy of certain correlated characters.

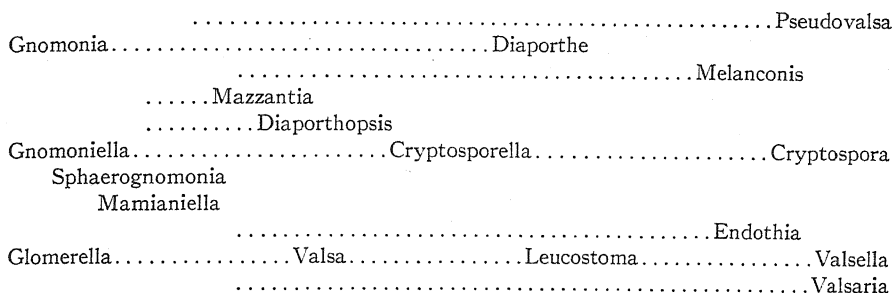
In the first series we have evidence of three diverging lines of development, made evident chiefly by differences in the septation and coloration of the conidia. The conidial fruit bodies are ectostromatic and bear their hymenium in enclosed locules, open cavities, or exposed layers. The conidia are of two types. One type remains cylindrical, one-celled, and hyaline throughout the series. The second type is one-celled and hyaline in *Diaporthe*, one-celled and colored in *Melanconis*, and hyaline or colored, but septate, in *Pseudovalsa*. The lower forms of these three diverging lines all have hyaline 2-celled ascospores, but the climax forms of each group show a distinctive type of ascospore. In *Diaporthe* the ascospores remain 2-celled and hyaline; in *Melanconis* they become 2-celled and brown; in *Pseudovalsa* they become many-celled and brown. Certain stromatic characters are also correlated with these differences in conidial characters and with the evolution of the ascospore, as already noted.

In the second series the ascospores are elliptical, hyaline, and one-celled to long-cylindrical, hyaline, and sometimes faintly septate. The imperfect fruit body is similar to that of the first series, but the conidia are all one-celled, hyaline, and somewhat elongated. A second type of conidium is present, at least in some species. The configuration of the perithecial stroma resembles that of *Melanconis*.

In the third series is found a set of characters which contrast it to both of the first two series. The ascospores are allantoid, one-celled, and hyaline to elliptical, 2-celled, and brown. The imperfect fruit body is either ectostromatic or entostromatic and consists of numerous labyrinthiform locules within a stroma. The conidia are small, allantoid to elliptical, one-celled, and hyaline. Only one type of conidium is known. The perithecial stromata are various in structure.

As the simple (non-stromatic) forms of the Diaporthaceae all have similar imperfect stages so far as known, the ancestral forms of these three series are suggested by the type of ascospore to be *Gnomonia*, *Gnomoniella*, and *Glomerella*, respectively.

The relationships of the Diaporthaceae may be represented diagrammatically as follows:



### SUMMARY

From the preceding discussion we may draw the following conclusions:

1. It is no longer necessary to construct the taxonomy of the stromatic Sphaeriales upon purely arbitrary and uncorrelated characters; instead, one finds that there exist definite phylogenetically related groups which can serve as a basis for classification. It should be pointed out here that the stromatic Sphaeriales do not themselves form a complete line of evolution, but represent merely the higher stromatic development of lower forms. The Diaporthaceae and the Allantosphaeriaceae should include, therefore, in a natural classification, the simpler forms; as they are included in von Höhnelt's outline of these families. The stromatic forms were chosen merely as a convenient group for intensive study.

2. After determining these phylogenetic relationships, we can formulate certain generalities in regard to the evolution of the group as a whole, and, therefore, as to its classification:

(a) In the first place the evolution of the group has been polyphyletic, consisting of a number of parallel series. These series should comprise, therefore, the larger groups in any classification, according to their rank in the evolutionary series.

(b) The development of the stroma and the compound fruit body is

common to all the evolutionary series within this group. This character, therefore, can not be used as a differential character for the larger divisions of a taxonomic system. The minor variations of stromatic development are valuable, however, when used in correlation with other characters or to determine relationships within one given series.

(c) In this group at least, the characteristics of the imperfect fruit body are definitely correlated with those of the perfect stage, and are very valuable characters for the determination of relationships. The conidial fructification often shows an evolution parallel to that of the perithecial fructification, as is seen in the coloration of the conidia of the *Pseudovalsa* series or in the elongation of the conidia in the *Cryptosporella*-*Cryptospora* series. Characters of the conidial fruit body should not be used in the separation of taxonomic divisions, however, without the presence of correlated differences in the perithecial fruit body, on account of the practical difficulty of determining the genetic connection of the two stages of the life history.

With the preceding principles in mind, let us see what changes their application will make in the previous classifications in this group. For the sake of clarity, let us set down three taxonomic systems, which will illustrate the chief variations in viewpoint as to the classification of this group.

I. That of Lindau, in Engler and Prantl's "Natürliche Pflanzenfamilien" (1897):

Valsaceae:

Anthostoma	Vialaea	Caudospora	Rhyncostoma
Diaporthe	Kalmusia	Thyridella	Thyridium
Fenestella			

Valsa

Sub-genera:	Eutypa	Cryptovalsa	Leucostoma
	Endoxyla	Cryptosphaerella	Eutypella
	Cryptosphaeria	Endoxylina	Valsella
	Euvalsa		

Melanconidaceae:

Cryptosporella	Valsaria	Melanconis	Calospora	Pseudovalsa
Cryptospora	Titania	Melanconiella	Holstiella	

Diatrypaceae:

Calosphaeriae:		Diatrypeae:	
Calosphaeria	Cacosphaeria	Diatrype	Quaternaria
Coronophora		Scoptria	Pleurostoma
		Diatrypella	

Melogrammataceae:

Gibellia	Botryosphaeria	Myrmaecium	Melogramma
Endothia	Myrmaeciella	Sillia	Berlesiella

## II. That of von Höhnelt (57, 59) given in 1918 (stromatic forms):

## Allantosphaeriaceen:

## Diatrypeen:

Cryptosphaeria  
Quaternaria  
Cryptovalsa  
Diatrypella

Eutypa  
Eutypella  
Diatrype

## Valseen:

Later transferred to the  
Diaportheen.

## Diaportheen:

## Eu-Diaportheen:

Mazzantia  
Diaporthopsis  
Cryptosporella  
Vialaea  
Cryptospora

Apioporthes  
Endothia  
Diaporthes  
Calosporella

## Valseen:

Valsa  
Leucostoma  
Valsella

(Scoptria and Pereneutypa,  
related to Eutypella)

## III. A tentative grouping upon the basis of the preceding outline of the writer's conclusions:

## Allantosphaeriaceae:

## Diatrypeae:

Eutypa	Diatrype	Diatrypella	Eutypella	Cryptosphaeria
				Valsaria ( <i>p.p.</i> )
				Anthostoma

## Diaporthaceae:

## Eu-Diaportheae:

Diaporthes	Diaporthopsis
Melanconis	Mazzantia
Pseudovalsa	Cryptospora
	Cryptosporella

## Valseae:

Valsa  
Leucostoma  
Valsella  
Endothia  
Valsaria (*p.p.*)

All the earlier systems of classification, from that of Nitschke (83) until the time of von Höhnelt's rearrangement of these genera, are modeled on a basis very similar to the system of Lindau given above. The four families used by all these workers are very indefinite and widely overlapping in their characters. There is practically no character representative of any of the families, and they are rather traditional groupings of genera than related forms. The family Melanconidaceae is fairly constant, containing those genera of the Diaporthaceae with open conidial layers. The genus Diaporthes, however, is placed in the Valsaceae, while Endothia and Sillia are placed in the Melogrammataceae. Of the genera of the Allantosphaeriaceae, Diatrype, Quaternaria, and Diatrypella are placed in the Diatrypaceae, Anthostoma as a genus of the Valsaceae, and the rest under Valsa as subgenera. Euvalsa, Leucostoma, and Valsella, which, as we have seen, belong to the Diaporthaceae, are also placed as subgenera of Valsa. It will be noticed that Lindau's list of genera includes a number not considered by the writer. Many of these are genera segregated or indefinitely differentiated from those discussed. Others are small genera, material of which

was not available to the writer. Later studies of such genera will determine their true position in the general scheme here presented.

The recognition by von Höhnelt of the two large groups, the Allantosphaeriaceae and the Diaporthaceae, was a long step in advance over all previous classifications.

The writer's results have pointed to relationships which follow the general outlines put forth by von Höhnelt. The chief changes which are suggested are as follows:

1. The union of the genera *Cryptovalsa* and *Diatrypella*, since they are merely arbitrarily separated portions of the same line of development, differing only in degree of stromatic development.
2. The inclusion of the genera *Endoxyla*, *Anthostoma*, and certain species of *Valsaria* in the Allantosphaeriaceae, for reasons already stated.
3. The placing of *Endothia* in the Valsaceae, on account of the character of its conidial stage and because of the presence of allantoid ascospores in two species. The position of *Valsaria* in the Valsaceae is more or less questionable until further evidence is accumulated.
4. The inclusion of *Melanconis* and *Pseudovalsa* in the Diaporthaceae, since they show a distinct phylogenetic relationship to Diaporthe.

#### PROVISIONAL DESCRIPTION OF GENERA

The following generic descriptions are given in an attempt to circumscribe the genera studied on the basis of the phylogenetic relationships already discussed. In some cases the evidence is as yet inadequate for any final separation of generic groups. In such cases (as in *Eutypa*) a wide range of forms have been included in a single genus. The writer realizes that such groupings present a composite genus, and will necessarily call for rearrangement as our knowledge of life histories progresses. The following descriptions, however, are merely provisional, and given to present more clearly the preliminary outlines of the phylogeny of this group.

#### ALLANTOSPHAERIACEAE v. H.

Asci with more or less elongated, tapering, persistent stalks, resulting in a persistent, definite hymenial layer of asci. Paraphyses present but usually evanescent at maturity. Ostioles characteristically sulcate, except in *Anthostoma*. Asci 8- to many-spored. Ascospores allantoid to cylindrical or inequilateral-elliptical, one- to many-celled, yellowish hyaline to brown.

Conidia long-cylindrical to filiform, curved or hamate, and one-celled, borne in exposed layers, open cavities, enclosed locules, or labyrinthiform chambers (on free conidiophores in *Eutypa*). Either ectostromatic or entostromatic.

#### *Eutypa* Tul.

Stroma effuse; perithecia separately erumpent. Ectostroma not strongly developed, or absent. Ostioles punctate to sulcate. Ascospores biserial, allantoid, yellow-hyaline, one-celled.

Imperfect stage ectostromatic, entostromatic, or hyphomycetous.

*Cryptosphaeria* Grev. *emend.*

Stromata effuse or isolated; perithecia separately or collectively erumpent, usually surrounded by a dark marginal zone. No mechanical ectostroma formed. Asci 8-spored. Ascospores irregularly biseriata, allantoid to cylindrical, brown, one- to many-septate, often constricted. Filiform paraphyses present, and more or less persistent.

Imperfect stage entostromatic, forming labyrinthiform locules, so far as known.

Since the type species of Greville's *Cryptosphaeria* (*C. millipunctata*) has septate ascospores, and since, according to the scheme here presented, the other species of *Cryptosphaeria* would fall in either *Eutypa* or *Euanthostoma*, the name *Cryptosphaeria* is retained for those species with septate brown ascospores.

The species of *Valsaria* with obviously allantoid spores are placed here. It may be found advisable later to erect a new genus for these species with clustered perithecia.

*Quaternaria* Tul.

Entostroma effuse, but perithecia in small collectively erumpent clusters of 2-4. Spores biseriata, allantoid, one-celled, yellow-brown.

Imperfect stage ectostromatic (*Libertella*).

This genus would differ from *Eutypa* only in its collectively erumpent perithecia, and would include some species usually placed in that genus. It would differ from *Eutypella* only in its ectostromatic conidial stage.

*Diatrype* Fr. *emend.*

Stroma effuse or isolated. Ectostroma strongly developed and deciduous. Entostroma forming a widely erumpent disc; dark marginal zone present. Perithecia lying parallel and separately erumpent. Ostioles sulcate. Asci 8-spored. Spores biseriata, allantoid, one-celled, yellow-hyaline.

Imperfect stage ectostromatic.

*Diatrypella* Ces. & de Not. *emend.*

Stroma effuse or isolated. Ectostroma absent or strongly developed, but not deciduous. Entostroma well developed, often pustulate but usually not widely erumpent, bounded by a dark marginal zone. Perithecia usually clustered, rarely separately erumpent. Ostioles usually sulcate. Asci long-stalked, polysporous. Spores allantoid, one-celled, yellow-hyaline.

Imperfect stage primarily ectostromatic.

*Diatrypella* and *Cryptovalsa* Ces. & de Not. are here included under one genus, since they represent merely arbitrarily chosen portions of the same line of development and show no sharp line of demarcation.

*Eutypella* Nit.

Stroma effuse or isolated. Perithecia clustered and collectively erumpent (in some species some of the perithecia are sometimes separately erumpent). Ectostroma limited in development, usually in isolated patches. Entostroma often well developed and pustulate, surrounded by a dark



marginal zone. Ostioles sulcate. Asci 8-spored. Spores biseriate, allantoid, one-celled, yellowish to brownish-hyaline.

Imperfect stage entostromatic (Cytosporina).

*Anthostoma* Nit. *emend.*

Stroma effuse or isolated. No ectostromatic development. Perithecia separately erumpent (Euanthostoma), or clustered and collectively erumpent (Lopadostoma). Entostroma often strongly developed and serving to burst open the periderm, with or without a dark marginal zone. Ostioles irregularly sulcate or merely punctate. Asci cylindric-clavate to cylindrical, short-stalked, 8-spored. Spores irregularly biseriate to uniseriate, brown, allantoid to cylindrical or usually inequilaterally elliptical, one-celled. Paraphyses numerous and persistent.

Imperfect stage entostromatic (so far as known).

This description would include the dark-spored forms of *Cryptosphaeria*, e.g., *C. vicinula*.

DIAPORTHACEAE v. H.

Asci with short, evanescent stalks, soluble in water, resulting in a loose mass of free asci and spores within the perithecium except in those cases in which the asci are held in position by numerous paraphyses. Asci usually with a protoplasmic ring in the thickened tip. Paraphyses present; sometimes few and evanescent; sometimes numerous and persistent. Ascospores variable; elliptical, fusoid, allantoid, or long-cylindrical, one- to many-celled, hyaline or colored.

Imperfect stage various. Mostly with two types of conidia; one of which is short-cylindrical to filiform, curved or hamate, hyaline and one-celled, while the other may be elliptical, fusoid, long-cylindrical to clavate, one- to many-celled, or hyaline to brown. Conidia borne in exposed layers, open cavities, enclosed locules, or labyrinthiform chambers. Either ectostromatic or entostromatic.

*Diaporthe* Nit. *emend.*

Stromata effuse or isolated. Entostromatic areas more or less differentiated and light in color. A blackening of the substratum always present, either on the surface of the substratum or as a marginal zone about the entostromatic areas. Paraphyses few and evanescent. Ascospores elliptical or fusoid, hyaline, 2-celled, sometimes appendiculate.

Imperfect stage belonging to the form genus *Phomopsis*. Conidia of two types, produced within enclosed locules which are usually surrounded by a wall-like zone of dark-colored cells. Ectostromatic or entostromatic. One type of conidium cylindrical or elongate-fusoid to filiform, curved or hamate, hyaline, and one-celled; second type elliptical to fusoid, hyaline, and one-celled.

The present species of *Diaporthe* which would not be included under this description would fall within the genera *Melanconis* and *Pseudovalsa* as subsequently described.

*Melanconis* Tul. *emend.*

Stromata, so far as known, isolated. Disc consisting of a well developed conical ectostroma. Perithecia arranged circinately in the unaltered bark

tissue. No entostromatic development apparent, and no dark zone present. Paraphyses present; sometimes fairly numerous and persistent. Ascospores biserial to uniserial, elliptical, 2-celled, hyaline or brown, sometimes appendaged.

Imperfect stage belonging to the form genus *Melanconium*. Hymenial layers formed over the entire surface, or within cavities upon the flanks of a conical or pulvinate ectostroma. Conidia of two types, at least in some species: one, small, cylindrical to elliptical, one-celled, and hyaline; the second, larger, elliptical to fusoid, one-celled, and colored.

*Pseudovalsa* Ces. & de Not. *emend.*

Stromata effuse (?) or isolated. Ectostroma not sharply differentiated; stromatic development usually entostromatic. Perithecia irregularly scattered or clustered. Entostromatic tissue usually dark-colored, not light in color as in *Diaporthe*. Irregular darkened marginal zones present in some species. Ascospores biserial, two- to many-celled, hyaline or brown, sometimes appendaged.

Conidia borne in enclosed locules, shallow cavities, or exposed layers, within or upon an ectostromatic tissue. Conidia of two types: one, small, long-cylindric to allantoid, curved or hamate, hyaline, one-celled; the second, larger, elliptical to clavate or elongate, hyaline or brown, one- to many-septate.

The above description has been made general enough to include all forms hitherto reported with septate conidia. As has been pointed out, the evidence for such a grouping is as yet insufficient, and further cultural connections are needed either to confirm or to alter these relationships.

*Mazzantia* Mont.

Stroma isolated. Entostroma differentiated, light in color, bounded by a darkened marginal zone. Ascospores one-celled, elliptical, hyaline.

Conidia cylindrical, hyaline, one-celled, within enclosed locules.

Further study may show it advisable to unite this genus with *Diaporthopsis* into a genus corresponding to *Diaporthe* but with one-celled ascospores.

*Diaporthopsis* Fabre

Stromata effuse. Entostromata differentiated, light in color, bounded by a dark marginal zone. Ascospores one-celled and hyaline.

*Cryptosporella* Sacc.

Stromata isolated. Ectostroma forming a small conical disc. Entostroma not developed; perithecia buried in the unaltered bark; no marginal line present. Ascospores elliptical to fusoid, hyaline, one-celled.

Conidia elliptical to fusoid, hyaline, one-celled, produced in more or less open cavities or locules within an ectostroma. Sometimes a second type of smaller, cylindrical or filiform, curved, hyaline, one-celled conidia.

*Cryptospora* Tul. *pro parte*

Stromata as in *Cryptosporella*. Ascospores long-cylindrical, hyaline, one-celled or faintly septate (*Sillia*).

Conidia elongate-fusoid or cylindrical, borne in open cavities or layers within an ectostroma. Sometimes a second type of smaller, filiform or cylindrical, curved, hyaline, one-celled conidia.

*Valsa* Fr. *pro parte* (section *Euvalsa* Nit.)

Stromata isolated. Perithecia clustered in the unaltered bark tissues beneath a distinct conical ectostroma. No marginal zone present. Ascospores allantoid, one-celled, hyaline. Asci 8-spored.

Pycnidia (*Cytospora*) entostromatic, containing numerous coalescent locules forming a labyrinthiform chamber. Conidia allantoid, one-celled, hyaline.

*Leucostoma* Nit. (section)

Stromata isolated or confluent. Each perithecial cluster enclosed by a dark marginal zone, within which there is a strong development of entostromatic hyphae. Perithecia imbedded within this differentiated entostroma. Asci 8-spored. Ascospores allantoid, hyaline, one-celled.

Pycnidia and conidia as in *Valsa*, but with a somewhat greater stromatic development.

*Valsella* Fck.

Same as *Leucostoma*, except that the asci are many-spored.

*Endothia* Fr. (sense of Shear)

Stromata isolated or confluent. Entostroma strongly developed, colored, without any marginal zone. Ascospores biserial, allantoid to elliptical, hyaline, one- or 2-celled.

Pycnidia consisting of numerous locules which often coalesce to form a labyrinthiform chamber, formed within either ectostromatic or entostromatic tissue. Conidia small, elliptical to cylindrical, hyaline, one-celled.

*Valsaria* Ces. & de Not. *pro parte*

Stromata isolated or confluent. Practically no ectostromatic development. Entostroma very strongly developed, often erumpent, bounded by a dark marginal zone, usually colored. Paraphyses numerous and persistent. Ascospores uniseriate, elliptic-fusoid, 2-celled, brown.

Pycnidia consisting of numerous or labyrinthiform locules within the entostroma. Conidia small, elliptical, one-celled, hyaline.

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## STUDIES ON ASTER YELLOWS<sup>1</sup>

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An infectious chlorosis known as "aster yellows," because it attacks the China aster, *Callistephus chinensis* Nees, is prevalent throughout the United States. The aster is grown extensively in other parts of the world, especially in Europe and the Orient, but the disease is known to be prevalent only in North America. Although it has been present here for many years and is a serious hindrance to aster growing, it has been given little attention by plant pathologists. This is partly, no doubt, because it belongs to that group of obscure plant maladies known as virus diseases. Another probable reason why pathologists have neglected aster yellows is that it is not known to attack any plant of great economic importance. Smith (26) gave a good description of the disease in 1902 and suggested that it might go to other plants in the Compositae closely related to the China aster. He was unable to find any parasitic organism associated with yellows and suspected its relationship to the virus disease group. Whether the incidence of aster yellows has changed during the past twenty-five years is not known. Smith speaks of it as "widespread and destructive." At the present time it is so serious in many sections of the country that the planting of asters is being greatly restricted or even abandoned. Aster plots showing 90 to 95 percent of yellowed plants are not uncommon throughout the eastern United States. One hundred percent infection has occasionally been observed in fields containing several hundred plants. In certain localities, however, the disease is not yet serious, and small plantings sometimes remain disease-free to the end of the season.

At the suggestion of Doctor William Crocker, the writer undertook a study of aster yellows in the spring of 1923. The work, started at that time, has been continued during the past three years. Special attention was given to the means by which the disease is transmitted to healthy plants, to its relationship to similar well known diseases of other plants, to its overwintering in wild host plants, to the life and habits of its insect carrier, to its incubation period in both plant and insect host, to its host range, to possible methods for its control, and to its etiology. Several brief reports covering certain phases of the work have already been published (12, 14, 15). The object of the present paper is to record in more detail observa-

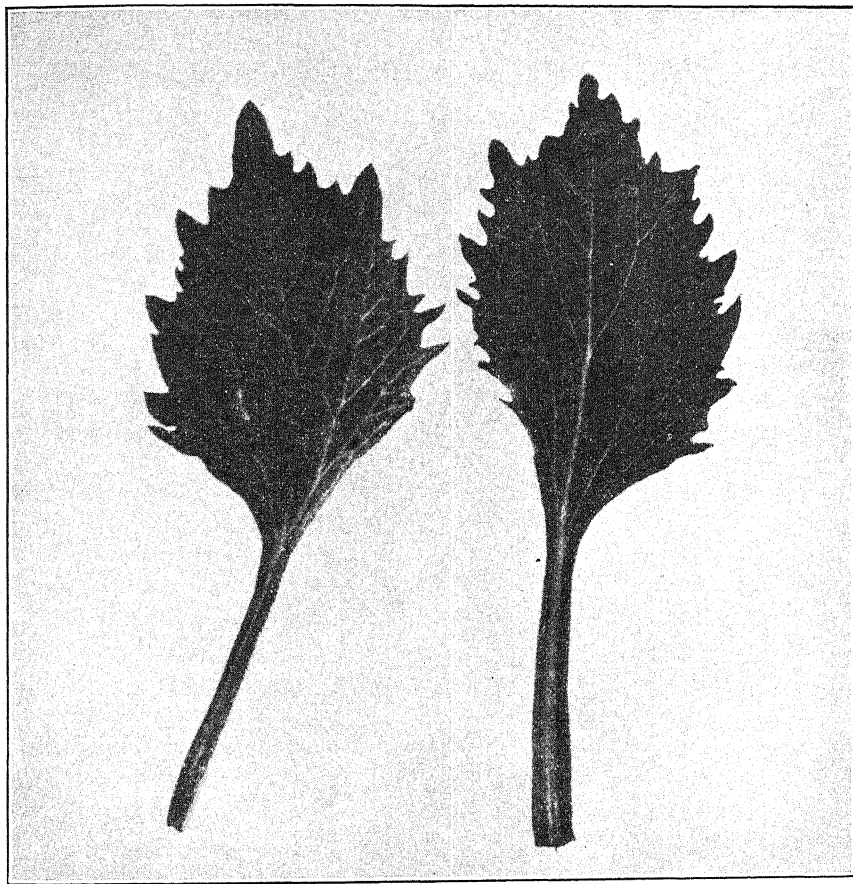
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tions and experimental results obtained to date and to discuss the relation of these data to the yellows-disease problem.

#### SYMPTOMS ON ASTER

Smith (26) has carefully described and pictured the yellows disease as he observed it on aster. A detailed description need not, therefore, be given here. A brief account of the development of the disease will, however, give opportunity to call attention to some of the more important symptoms.

Aster leaves affected with yellows never show mottling. The disease is, therefore, easy to distinguish from chloroses of the mosaic type. In advanced stages yellows is always systemic in the above-ground portions of the



TEXT FIG. 1. Showing "clearing of the veins" in affected leaves.

plant. The first symptom to be observed on a young plant is a slight yellowing along the veins in the whole or in a part of a single young leaf. This symptom, which will be referred to as "clearing of the veins" (text

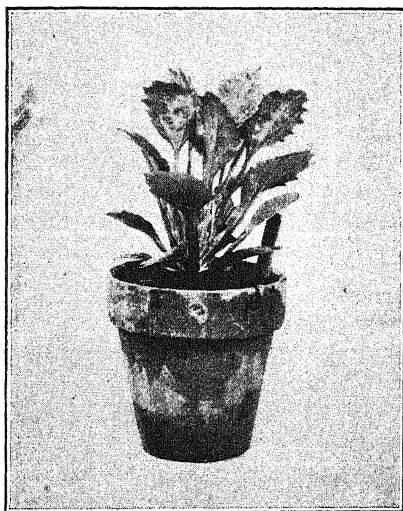
fig. 1), has not been observed in connection with any other aster disease and makes diagnosis possible long before conspicuous chlorosis appears. As more leaves develop shortly after infection, they show the same clearing of veins. After a plant has been diseased for some time the new leaves are chlorotic throughout. The larger veins seem to be a barrier to the spread of yellows in leaves. The tissues on one side of a midrib or of any large leaf vein are frequently chlorotic while those on the other side are of a normal green color. One half or more, or a sector amounting to less than one half of a plant, may be chlorotic for some time before the remaining portion is affected. This shows that rapid spread around the stem of the plant does not take place in many cases. It is probable that such sectorial infections occur when the disease enters the stem some distance back of the apical bud and when longitudinal growth of the stem is approximately equal to the rate of spread of the disease. This is also indicated by the fact that lateral shoots sometimes show the disease before it appears in the terminal portions of the stem. While chlorosis does not appear in leaves that are mature when the plant is attacked, leaves that are from one half to two thirds full grown and are of a normal green color do become diseased, as is shown by clearing of veins and general chlorosis. This fact shows that the disease is capable of destroying chlorophyll. It is probable that it also depresses the production of chlorophyll in leaves developed from diseased buds. When the attack is severe the young leaves are almost white. Such leaves may become more or less green as they grow old. The disease causes chlorosis in all green portions of the plant. Strangely enough, however, petals which normally contain no chlorophyll become quite green when diseased. While yellows depresses chlorophyll-production in portions of the plant that are normally green, it causes the production of some green-colored substance in floral parts where chlorophyll is not normally present. Since this green substance is present in the cell sap, it is probably not chlorophyll. In different plants and under different conditions the disease causes widely different degrees of fading or yellowing of leaves varying from slight to extreme chlorosis. Some aster plants show much less chlorosis and are less stunted than others. They seem to resist the disease to a certain extent.

One of the most striking symptoms of aster yellows results from the abnormal production of secondary shoots. Such shoots frequently arise in the axils of leaves that show a normal green color and were mature before the plant became infected. They are always thin and have the appearance of etiolated branches.

Plants that contract the disease before they are mature are always more or less stunted. The degree of stunting varies with the age of the plant at the time it becomes infected and with the size of the sector infected. The effect of the disease on the main stem is greatly to shorten the internodes. It usually has the opposite effect on the secondary shoots. Diseased flowers and seeds are often much larger than healthy ones, but cases also

occur in which they are dwarfed. The flower heads are always more or less dwarfed. Individual flowers in some cases develop into vegetative branches, which may or may not bear small flower heads. Trichomes on diseased flowers frequently develop into leaf-like structures. The petals of ray flowers are uniformly much dwarfed. The root systems of diseased plants appear normal but are smaller than those of healthy plants.

Another interesting result of yellows is the change which it brings about in the response of the plant to gravity. Instead of diseased leaves lying flat and making a broad angle with the vertical, they stand upright, as is shown in text figure 2. A similar response is shown by individual flowers



TEXT FIG. 2. Upright position of leaves in affected plants.

and branches of diseased plants. This modification of the response of plants to gravity sometimes occurs in the case of certain plants infected with rust and smut diseases where mycelia are systemic.

Yellows also causes certain morphological changes. Diseased leaves are frequently somewhat deformed. Their petioles are longer than those of healthy leaves of the same age. The leaf blade on the other hand is narrower, and on the whole smaller, than the normal blade. Diseased leaves may have deep clefts and notched margins but are seldom severely deformed.

Necrosis is a symptom of advanced stages of yellows. It is most severe in the stem tissues a short distance below the apical bud of the main stem or of a branch. It causes the collapse of certain tissues but does not kill the plant. Diseased plants that escape infection by fungi will live as long, or almost as long, as healthy ones.

In summarizing, it may be said that the yellows of asters is an infectious disease causing diffuse and well marked chlorosis, clearing of veins, occasional

one-sided or sectorial infection, upright habit of growth, malformation and increased growth in certain organs, dwarfing of the plant as a whole, and the abnormal production of secondary shoots.

#### INSECT TRANSMISSION

Because of the similarity between aster yellows and certain insect-borne virus diseases, such as curly-top of beet, sugar cane and corn mosaic, the writer was led to suspect that its spread might be due to insects. Observations made on aster plots at the Boyce Thompson Institute at Yonkers, New York, during the summer of 1923 showed that at the time the disease was spreading rapidly the tarnished plant bug, *Lygus pratensis* L., and two leafhoppers, *Cicadula sexnotata* Fall., and *Empoasca flavescens* Fab., were present in large numbers. Two other leafhoppers, *Agallia sanguinolenta* Prov. and *Graphocephala coccinea* Forst., were present but not abundant. Aphids were found in small numbers but were never numerous. The same insects were present in other aster fields in the vicinity of New York City. Because of their activity and abundance, leafhoppers and the tarnished plant bug were suspected of spreading the disease.

In order to test the insect hypothesis of transmission, a number of experiments were undertaken. The aster variety, Late Branching, obtained from Vicks' Sons of Rochester, New York, was used in all experiments here reported.

#### Experiments with the Tarnished Plant Bug, *Lygus pratensis* L.

Ten adult tarnished plant bugs were transferred from diseased aster plants to each of 3 insect-proof cages. Each cage contained 4 healthy and 2 yellowed aster plants. Four healthy and 2 yellowed aster plants in another insect-proof cage served as checks. The bugs lived and reproduced in each of the 3 cages to which they were transferred, but the 12 healthy plants in these cages as well as the 4 healthy check plants remained healthy for 3 months, when the experiment was ended.

Ten adult tarnished plant bugs were transferred from diseased aster plants to each of 4 insect-proof cages. Each cage contained 6 healthy young aster plants. Twelve other plants of the same age growing in 2 other cages served as checks. The bugs lived and reproduced in each of the 4 cages to which they were transferred, but all plants remained healthy during the 3 months that they were kept under observation.

After being compelled to feed for one week on yellowed aster plants in insect-proof cages, 25 tarnished plant bugs were transferred to each of 3 insect-proof cages containing 2 yellowed and 3 healthy young aster plants. The bugs lived and reproduced. They caused considerable injury to the plants through their punctures, but all the healthy plants remained healthy during the 3 months that they were kept under observation.

Ten tarnished plant bugs reared on yellowed aster plants in insect-proof

cages were transferred to each of 7 insect-proof cages containing 6 healthy young aster plants each. The bugs lived and reproduced in each of the cages, but all plants remained healthy during a period of a little more than 3 months that they were kept under observation.

Five tarnished plant bugs reared on yellowed aster plants were transferred to each of 3 insect-proof cages containing 6 healthy young aster plants each. The bugs lived and were observed to feed, but all plants remained healthy during the 3 months they were kept under observation.

These negative results with the tarnished plant bug together with confirmatory field evidence indicate that this insect is unable to transmit aster yellows.

### Experiments with the Green Aster Leafhopper, *Empoasca flavescens* Fab.

A small green leafhopper closely resembling the potato leafhopper, *Empoasca fabae* (Harr.), was abundant on asters in the vicinity of New York City during the past three seasons. It was submitted to several authorities on this group of insects for identification. They did not agree in their opinions regarding the species to which it belongs. Prof. Herbert Osborn, to whom specimens were sent, reported that it is probably *Empoasca flavescens* Fab. His opinion is in agreement with that of Dr. Albert Hartzell, who carefully compared it with other species of *Empoasca*. This identification has, therefore, been accepted.

Although the green aster leafhopper closely resembles the potato leafhopper, it seems to be distinct from this species for the following reasons. Leafhopper cultures taken from asters and from potatoes grown in close proximity in the same field prove that the green aster leafhopper does not produce colonies on the potato and that the potato leafhopper does not live and breed on the aster. The aster leafhopper, when forced to feed on potato plants, produces a whitish stippling on the leaves just as it does on aster leaves. The potato leafhopper does not produce such stippling either on potato or on aster. Moreover, the potato leafhopper when grown on potato plants causes the disease known as hopperburn, while the aster leafhopper when cultured in the same way causes no hopperburn.

The green aster leafhopper was first cultured on asters in insect-proof cages in July, 1923. Twenty-five adults were transferred from plants growing in field plots where yellows was abundant to each of 5 insect-proof cages. Each cage contained 6 healthy young aster plants. Six healthy young plants of the same age kept in a similar cage served as checks. The leafhoppers thrived and reproduced in each of the 5 cages, but the 30 plants on which they fed as well as the 6 check plants remained healthy during the 3 months the experiment was in progress.

The green aster leafhopper was kept in culture on aster plants in insect-proof cages for a little more than 2 years. It reproduced continuously during this period, passing through many generations. The colonies

flourished in the winter as well as in the summer. Insects reared on yellowed aster plants were transferred at different times to healthy young plants grown in cages. Several hundred plants were exposed in this way during the 2 years this leafhopper was cultured. From 25 to 300 adults reared on yellowed plants were kept for varying periods of time on healthy young aster plants. The details of these experiments will not be given, as all results were negative. The green aster leafhopper seems unable to transmit the disease. Similar but less extensive experiments were made in attempts to transmit yellows to aster by means of the potato leafhopper, *Empoasca fabae*. This leafhopper also failed to transmit the disease.

#### Experiments with the Clover Leafhopper, *Agallia sanguinolenta* Prov.

In March, 1924, many blocks of frozen soil containing rye plants from seed sown the previous autumn between rows of aster plants were brought into a warm laboratory. The object of this experiment was to determine, if possible, whether any of the aster leafhoppers live over winter in the adult stage. It was hoped that if adults were present in the soil around the rye plants they would become active when brought into the laboratory. Only one species of leafhopper, *Agallia sanguinolenta*, was obtained in this way. The blocks of soil were put into flats and each placed near a window. The plants and the windows were examined twice daily for the presence of leafhoppers. After from 1 to 3 days, adults of the clover leafhopper were found on the soil in the flats or on the windows above the flats. No other leafhopper was obtained. Many blocks of soil failed to yield any leafhoppers, but a total of 13 adult clover leafhoppers were secured. It is interesting to note that all these adults were of exactly the same size and somewhat smaller than adults of the species present during the summer. This observation suggests that only those individuals that moult for the last time just before cold weather sets in, in the fall, are able to live through the winter. The 13 clover leafhoppers obtained in this way were placed on healthy young aster plants. They reproduced, and from them a strong colony was obtained. It was kept in culture on asters for a little more than one year. During this time it passed through many generations. Suitable colonies reared on yellowed aster plants were repeatedly transferred to healthy aster plants, but in no case did they transmit the yellows disease. Although the clover leafhopper flourishes on aster, it was finally concluded that it takes no part in the spread of yellows.

#### Experiments with the Peach Aphid, *Myzus persicae* Sulz.

This aphid is seldom found in large numbers on field-grown aster plants, but the *Calendula*, which is very susceptible to aster yellows, is one of its favorite hosts. If infested *Calendula* plants are caged with aster plants, the aphids will spread to and multiply on the asters.

Fifty peach aphids, consisting of both adults and nymphs taken from

yellowed Calendula plants, were placed on 6 healthy young Calendula plants in an insect-proof cage. At the same time, 25 similar aphids were placed on each of 6 other healthy young Calendula plants in another cage. Six other healthy Calendula plants of the same age kept free of insects served as checks. All plants remained healthy during the 2 months that they were kept under observation.

Approximately one half cubic centimeter of peach aphids, consisting of both nymphs and adults taken from a yellowed aster plant which had been infested for a period of 2 weeks, were placed on a healthy young aster plant. After a period of 2 weeks, all aphids were killed by fumigation. The plant remained healthy for 2 months, when the experiment was ended.

Four cubic centimeters of peach aphids taken from yellowed aster plants were placed on 12 healthy young aster plants in an insect-proof cage. At the same time the same quantity of peach aphids from the same source was placed on 4 other healthy young aster plants in a second cage. The aphids were allowed to feed on the exposed plants during 6 weeks that the experiment was in progress. All plants remained healthy.

Approximately one half cubic centimeter of peach aphids reared on yellowed Calendula plants were transferred to each of 12 healthy young Calendula plants, and to one healthy young aster plant. The plants were kept in separate lantern-globe cages. Twelve healthy Calendula plants of the same age kept in a large cage served as checks. Five days after the experiment was started all insects were killed by fumigation. All the plants remained healthy for a period of 2 months, when the experiment was ended.

The above-described experiments bring evidence that the peach aphid is unable to transfer yellows to either of the two very susceptible host plants, aster and Calendula.

#### Experiments with Other Insects and with Mites

Yellowed aster plants infected with the greenhouse thrip, *Thrips tabaci* Lind. with the white fly, *Trialeurodes vaporariorum* W., and with the red mite, *Tetranychus telarius* L., were on several different occasions placed in cages containing healthy young aster plants. These insects and the mites always spread to the healthy plants, but in no case was yellows transmitted.

#### Experiment with the Leafhopper, *Graphocephala coccinea* Forst.

The beautiful large leafhopper, *Graphocephala coccinea*, occurs in small numbers on the aster. During the summer of 1923, 10 adults caught from yellowed plants in aster plots where yellows was abundant were transferred to a cage containing 6 healthy young aster plants. Six of these insects were still alive after 2 months, but they did not reproduce. All plants remained healthy during the 2 months they were kept under observation. Since this leafhopper was present in small numbers and did not reproduce on asters, it was not used in later experiments. While the experiment does not prove



that this *Graphocephala* can not transmit yellows, it at least shows that the insect does not readily transmit the disease.

#### Experiments with the Leafhopper, *Cicadula sexnotata* Fall.

Adults of this species were first observed by the writer on asters late in May, 1923. By the end of July they were present in considerable numbers. As it was evident that the aster is one of its favorite host plants, experiments were undertaken to test its relation, if any, to the spread of aster yellows.

On July 31, 25 adults taken from an aster plot containing many yellowed plants were placed in each of 2 insect-proof cages containing 6 healthy young aster plants each. One week later, 50 adults taken from the same aster plot were placed in each cage. The cages were numbered 1 and 2. The insects lived and reproduced in both cages. A third cage containing 6 similar healthy young plants served as a check. Eighteen days after the experiment was started, 3 of the plants in cage 2 showed yellows. Eight days later, a fourth plant became diseased. By September 10, all 6 plants were yellowed. On this date 2 healthy young plants were placed in the cage. On October 1, one of these plants showed the disease. Six days later the other plant became yellowed. Thirty-one days after the experiment was started one plant in cage 1 showed the first symptoms of yellows. The other 5 plants and the 6 check plants remained healthy during the two and one half months the experiment was in progress. The experiment shows that *Cicadula sexnotata* transmits the aster yellows disease.

In a second experiment, started August 9, 10 adults of this species were placed in cage 4 containing 6 healthy young aster plants. Twenty-five adults were placed in cage 5, which also contained 6 healthy young aster plants. The insects were caught in an aster plot containing many yellowed plants. Six healthy young aster plants in another cage served as checks. The leafhoppers flourished and reproduced in both cages. Eighteen days after the experiment was started one of the plants in cage 5 showed yellows. Three weeks later a second plant became diseased, and by September 3 a third plant was diseased. When the disease first appeared in this cage, 20 healthy young seedling aster plants were placed in the cage. Four weeks later 7 of the 20 seedlings showed yellows. Three of the large plants and 13 of the small seedlings remained healthy to the time the experiment was ended. The 6 plants in cage 4 and the 6 check plants remained healthy during the two and one half months the experiment was in progress. The insects in cage 5 transmitted the disease while those in cage 4 did not.

The leafhoppers lived and reproduced in the 4 cages in which they were placed. One hundred adults taken from cage 2, in which all plants had become diseased, were placed in cage 7 containing 31 healthy young plants. One hundred adults taken from cage 4, in which all plants had remained healthy, were transferred to cage 8 containing 32 other healthy young plants. The insects thrived in both cages. The experiment was started October 11.

By November 20, 40 days after the experiment was started, 19 of the 31 plants in cage 7 had yellows. One week later all the 31 plants showed the disease. The 32 plants in cage 8 remained healthy. The experiment shows that the colony of insects from cage 4 does not transmit yellows while that from cage 2 readily transmits it to healthy plants.

From December, 1923, to March, 1924, several transmission experiments in which *Cicadula* was used were carried out under carefully controlled conditions. In these experiments 183 aster plants were exposed to leafhoppers which had previously fed on yellowed aster plants. Of these plants 142 became diseased while 41 remained healthy. Fifty-eight check plants exposed to leafhoppers that had not fed on diseased asters did not take the disease.

The above-described experiments prove that *C. sexnotata* readily transmits aster yellows from diseased to healthy aster plants. They also show that colonies that have never fed on yellowed plants are unable to cause the disease.

*Cicadula sexnotata* Fall.

The small gray leafhopper found in abundance in aster fields in the vicinity of New York City was identified by several authorities to which it was submitted as *Cicadula sexnotata* Fall. It is present in North America from Alaska to the southernmost borders of the United States. It is also widespread and common throughout Europe. It occurs in Japan and probably throughout the Orient. In what part of the world it is endemic is not known. There is some evidence that the species has been introduced into North America. It was first reported in the United States by Forbes in 1884. Professor Herbert Osborn (21) found a specimen in the Harris collection of the Boston Society of Natural History which he thinks was collected between 1840 and 1850. Because several collectors failed to report its presence prior to the early eighties, Professor Osborn suggests that it may be an introduced species. He thinks it unlikely that this leafhopper could have been missed by such early collectors as Say, Fitch, and Uhler, if it had been so widespread and abundant as it is at the present time. It seems probable that it was introduced into this country less than one hundred years ago. It has been a common insect here for almost fifty years. Since aster yellows is not known in Europe, it was thought advisable to determine whether the *C. sexnotata* of Europe is identical with that prevalent in the United States. Specimens from cultures used in the experiments here reported were sent to Dr. A. D. Cotton of the Royal Botanic Gardens at Kew, England, with the request that they be compared with English specimens of the species. Dr. Cotton sent the specimens to the Keeper of Entomology at the British Natural History Museum at London. They were compared with English specimens by Mr. China, who reports that he can find no specific distinction between the British and the American specimens. A slight difference in color markings was observed, but, con-

sidering the variability in this respect of the British forms, Mr. China states as his opinion that the slight difference indicates at most only a slight variation. The writer is indebted to Dr. Cotton, Mr. China, and the Keeper of Entomology of the British Natural History Museum for this comparison and statement.

One of the reasons why this species of *Cicadula* is so abundant is that it feeds and breeds on a wide range of host plants. Osborn (21) reports it from grasslands and oat fields of Maine and mentions it as a serious pest on wheat, oats, and barley crops in the northwest (19). It is said to have caused serious damage to wheat in the province of Östergötland, Sweden, in the summer of 1918 (7). From Bohemia it is reported as injurious to sugar beets (31). It is well known throughout Germany as the cause of serious injury to grasses, cereals, and certain leguminous plants (10).

Osborn has given a careful description of its life history together with drawings of its eggs, of the five instars of nymphs, and of the adult (20, 21). Photographs of an adult and of nymphs in the first, second, and fifth instars are shown in figures *A*, *B*, *C*, and *D*, Plate XL. The only point in the life history of this insect which is not clear is its manner of passing the winter. This is a matter of considerable importance in connection with the overwintering of the aster yellows disease.

During the past three years the writer has kept this leafhopper in culture continuously in insect-proof cages. Twenty-five adults caught July 31, 1923, furnished the beginning of these cultures. No serious difficulty has been experienced in maintaining the cultures in a vigorous condition during all seasons of the year, except on one occasion when the temperature of the greenhouse in which the cages were kept went too low for a short period of time. The chief factor to be given attention is that a proper balance be maintained between number of insects and number and size of host plants. The cultures must also be kept free of insect enemies and fungous parasites. During the 3 years that *Cicadula* has been cultured it has passed through at least 25 generations. The females deposit eggs over a long period of time. Those of one generation are still depositing eggs when the females of the next generation start laying. No evidence has been found of distinct broods either in the field or in cultures. In a greenhouse kept at a temperature of 70° to 75° F. it will pass through its life cycle from egg to egg in about 40 days. The maximum or even the average number of eggs laid per female is not known. One female kept under careful observation during her life of 87 days laid eggs from which were hatched 127 nymphs. The average age reached by insects kept under favorable conditions at a temperature of 70° to 75° F., when only a few insects are kept in each culture and suitable food plants are supplied at least once a week, is about 120 days. When large numbers are kept in each cage the average length of life is not more than 60 days, even when great care is taken to provide suitable food plants at frequent intervals.

There is considerable variation in the length of time necessary for the eggs to hatch. In a culture kept in constant subdued light at a constant temperature of 68° F. and a relative humidity of 80 percent of saturation, the minimum period required for eggs to hatch proved to be 10 days. Under ordinary greenhouse conditions at temperatures ranging from 70° to 75° F., some eggs hatch in from 10 to 11 days after deposition while others require varying periods of time up to 3 weeks.

The eggs are deposited under both the upper and the lower epidermis of aster leaves. Some are deposited in the leaf petioles and even in branches and main stems. Most of them, however, are placed between the lower epidermis and the mesophyll of leaves. The eggs usually lie with their long axes in a plane parallel to the leaf surface, but occasionally they are placed obliquely to the leaf surface and lie with the distal end deep in the mesophyll. A waxy substance of a gray color plugs the hole made in the epidermis of the leaf when the egg is deposited. The eggs are frequently found in groups of three or four, but more often they occur singly. Plant cells adjoining the eggs remain turgid and normal in appearance from the time the eggs are deposited until they hatch. Eggs deposited in plants kept constantly in the dark failed to hatch. Whether this failure is due to the direct effect of darkness on the eggs or indirectly to some effect on the plant tissues in which they are deposited is not known.

In cultures kept at about 70° F. most of the eggs hatch in from 11 to 13 days. Most of the nymphs are in the second instar by the 16th day. By the 27th day some are in the fifth instar but many are still in the third and fourth instars. The first adults usually appear on the 31st day. Many are adult by the 33d day, but at least 10 more days must elapse before all insects reach the adult stage.

Both nymphs and adults vary considerably in color in cultures and in the field. Some are almost black while others are a light greenish-gray color. Ten dark-colored adults were collected and placed together in a culture. Their progeny contained no more dark-colored individuals than the progeny from light-colored adults.

*Cicadula sexnotata* will live and reproduce on a very large number of different host plants. Some of its favorite hosts are: aster, lettuce, sow thistle, great ragweed (*Ambrosia trifida* L.), daisy fleabane (*Erigeron annuus* (L.) Pers.) and other *Erigerons*, English plantain (*Plantago lanceolata* L.), dandelion (*Taraxacum officinale* Weber), wheat, oats, rye, barley, Calendula, *Ammobium alatum*, *Matricaria alba*, *Centaurea imperialis*, *Gaillardia grandiflora*, Moon Penny daisy (*Chrysanthemum leucanthemum*), and the African daisy (*Dimorphotheca aurantiaca*). Some plants on which they do not flourish are tobacco, potato, tomato, Schizanthus, peach, Begonia, and alsike clover. There are many plants on which they can live and reproduce, but on which they will not congregate when more favored host plants are available. Some examples of these are corn, African marigold, milkweed (*Asclepias nivea*), and cosmos.

An effort was made to determine how *Cicadula sexnotata* passes the winter. The method, already described, by which the clover leafhopper was shown to live over winter and was obtained from blocks of frozen soil in March, 1923, failed to yield a single specimen of *Cicadula*. Since it was present in large numbers in the field from which the blocks were taken, it should have been obtained by this method if it had been able to live through the winter. Both adults and nymphs, when subjected to temperatures of 5° C. or lower, die in a few hours. All evidence obtained indicates that they are unable to live through the winter.

On March 13, 1926, while the weather was quite cold, small blocks of soil containing a number of rye plants grown from seed sown the previous autumn were transferred to each of 8 different lantern-globe cages. Other blocks of soil with similar rye plants were placed in each of 2 flats and put into a large insect-proof cage. All cages were kept in a greenhouse. The lantern globes were not removed and the door of the large cage was never opened until the plants had been caged for 25 days. Each culture was then carefully examined. Eleven nymphs of *C. sexnotata* were found on the plants in the large cage. The 8 lantern-globe cultures were carefully examined and gave the following results: Culture number 1 contained two adults; culture number 2 was insect-free; culture number 3 contained one nymph in the fourth instar; culture number 4 contained one nymph in the second and one in the third instar; culture number 5 contained two nymphs in the fourth instar; culture number 6 contained one nymph in the fourth and one in the fifth instar; culture number 7 was insect-free; culture number 8 contained one nymph in the first instar.

No leafhoppers were obtained from similar rye plants held in a warm room for 4 days. This indicated that neither nymphs nor adults were present in the soil or on the plants when taken from the field. The insects that were present after 25 days could not have come from any source except the soil or the rye plants. While eggs were not actually observed in the rye plants, the experiment brings indirect proof that the species passes the winter in the egg stage. All nymphs obtained in the cultures used in the above experiment were kept to maturity and identified as belonging to *Cicadula sexnotata*.

The feeding habits of *Cicadula* were carefully studied. Insects caught in the field are wild and difficult to observe closely, but insects reared in lantern-globe cultures and transferred frequently become so tame that their bodies can be lifted by means of a needle without causing them to stop feeding. They try to feed on any portion of a leaf on which they alight. If, however, they fail to find a leaf vein they soon move to another place. After a suitable vein has been found they will frequently continue to feed in one place for more than an hour. As they pierce the tissues the posterior end of the body is sometimes moved sidewise through an angle of as much as 180°. This movement apparently aids the insect in boring into the tissues.

If large numbers of insects are placed on an aster plant, their feeding will cause wilting, discoloration, and other symptoms of injury. The severity of this effect depends on the size and age of the plant and on the number of insects. Plants soon recover from such direct effects if the insects are promptly removed. That this type of injury has no relation to the aster yellows disease is shown by the fact that virus-free as well as virus-bearing insects are equally effective in producing it, while only virus-carrying insects are capable of causing the yellows disease. Moreover, direct injury appears in a few hours after plants are exposed and is proportional to the number of insects and to the time during which they feed. Aster yellows on the other hand does not appear until after a definite incubation period averaging from 16 to 18 days, and its severity is in no way proportional to the number of insects used in transmitting it or to the time during which they feed.

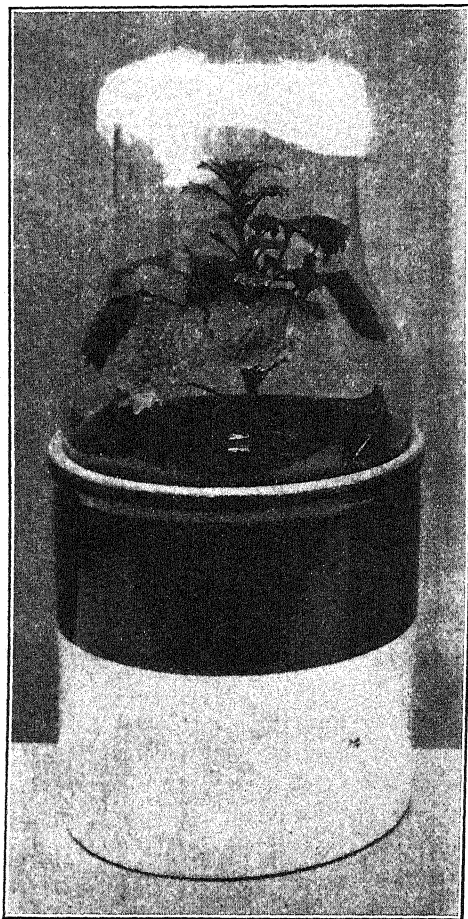
#### MATERIALS, APPARATUS, AND METHODS

In the experiments described below, vigorous young aster plants of the variety Late Branching were used except where otherwise stated. Insects from vigorous colonies were employed in all tests.

The experiments carried out during the first year of work were conducted in large insect-proof cages. During the second and third years the large cages were used for stock cultures only. They were not used in transmission experiments. It was found impossible to prevent entirely the escape of insects when the doors of these cages were opened for the purpose of watering plants or of removing plants or insects. For this reason a lantern-globe cage such as is shown in text figure 3 was used in all transmission experiments conducted during the past two years. The lantern-globe cages were covered with cheesecloth or with copper-wire cloth having fifty meshes to the inch. They were sometimes set over plants grown in large crocks. More often, however, they were set on glass plates over 6-inch pots containing young plants. During the time of exposure the caged plants were kept in a greenhouse. Insect transfers were made only in the laboratories. Virus-free insects were transferred in one laboratory; virus-bearing insects in another. The lantern-globe cages containing insects were never opened except in the laboratories. They were always opened before windows having a northern exposure. As *Cicadula sexnotata* is strongly attracted by light, any insect that escaped when a cage was opened went to a window. It could then be caught and placed in a tube with other members of its colony.

A transfer tube consisting of a glass test tube 8 inches long and about one inch in diameter was used for confining insects temporarily during the transfers. The test tube was fitted with a cork or rubber stopper through which a small glass tube, open at both ends, was inserted. The small tube was about 3 inches long and one half inch in diameter. It was placed so as to extend approximately one inch below the small end of the stopper. Insects were conducted into the large test tube through the small open tube.

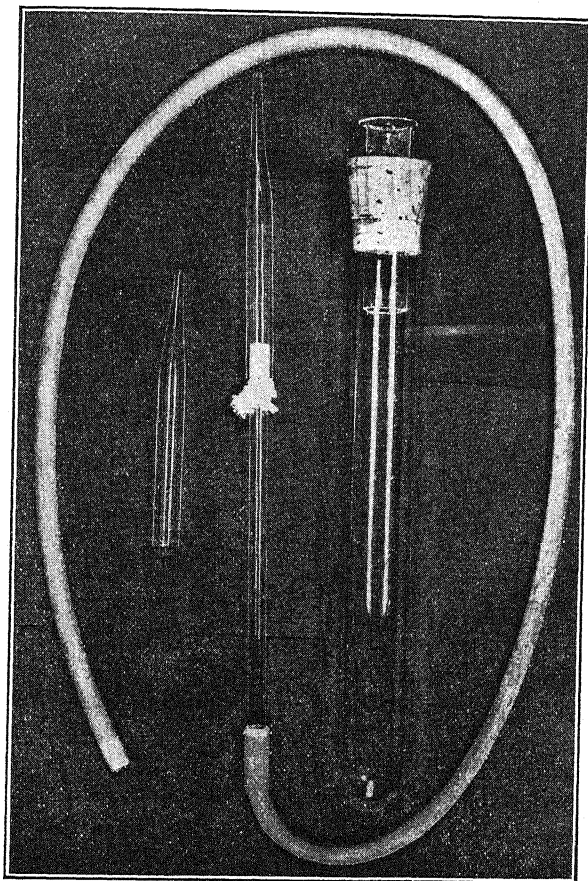
They do not readily escape from such a tube but may be removed when needed by means of an insect catcher. In case they were confined for a long period of time, a loose-fitting cotton plug was placed in the small tube.



TEXT FIG. 3. Showing method by which insects are confined to plants exposed to aster yellows.

All insect transfers were made by means of an insect catcher. This is a simple device made as follows. A piece of rubber tubing about 30 inches long and five sixteenths of an inch in outside diameter was fitted over one end of a small piece of glass tubing 5 inches long and one fourth of an inch in outside diameter. Two small pieces of cheesecloth were placed over the other end of the small glass tube, making a cap. One end of a second glass tube about 5 inches long and nine thirty-seconds of an inch inside diameter was fitted over the cloth-capped end of the smaller tube.

The other end of this tube was heated and drawn out, so as to have an inside diameter of approximately three thirty-seconds of an inch. It is important that the two glass tubes be of such diameter that the one over which the cheesecloth is placed fits easily yet snugly into the other. Both the transfer tube and the insect catcher are shown in text figure 4. When catching leafhoppers the free end of the rubber tube was placed in the



TEXT FIG. 4. Transfer tube and insect catcher.

mouth. The end of the glass tube having the small opening was placed near the insect to be caught, and air was quickly sucked through the tube. In this way insects are drawn through the small opening on to the cheesecloth. They are blown out by forcing air through the tube in the opposite direction. The catcher and transfer tube are convenient for catching and transferring, from cage to cage, leafhoppers, aphids, and other small insects. With this apparatus the writer was able to catch and transfer to a large



number of different lantern-globe cages as many as 2,800 leafhoppers in one day without the escape of a single insect.

In experiments on the transmission of yellows, plants were exposed to definite numbers of insects for definite periods of time. At the end of the period of exposure the insects were removed. Some insects were often lost by death during this time. The dead insects could usually be found, but in some cases, especially when the period was long, it was difficult to locate them. In order to be sure that no living insects remained on the plants after the termination of their exposure, they were fumigated with hydrocyanic acid gas immediately after being taken from the cages. Such fumigation readily kills leafhoppers. All exposed plants were fumigated at intervals of a few days during the time they were kept under observation in order to kill any insects hatched from eggs deposited during the exposure.

### Virus-free Colonies

It has been necessary for experimental purposes to maintain virus-free colonies of insects. These have been obtained in a number of different ways, as follows: (1) by rearing colonies from virus-free females captured early in the spring; (2) from insects hatched from dead rye and aster leaves; (3) from insects hatched from rye or wheat plants; (4) from insects hatched from aster plants that escaped infection; and (5) from insects hatched and taken from aster plants before yellows appeared.

On June 18, 1924, adults caught from lettuce growing in a garden were caged separately on 50 different healthy young aster plants. All the 50 plants remained healthy. Twenty-eight of the insects were females, and each produced a numerous progeny. All these colonies were maintained on aster plants for a period of 3 months. They all proved to be virus-free.

Before it was known that virus-free colonies could be reared from virus-carrying parents caged on wheat or rye plants, efforts were made to hatch eggs from dry leaves and leaf sheaths of rye plants and from dead aster leaves. The dry rye and aster leaves containing eggs were removed from the plants that bore them and placed on moist soil around healthy aster plants kept in cages. Many of the eggs in these dry leaves hatched. The nymphs went to the healthy aster plants and gave rise to colonies that always proved to be virus-free.

The third method of obtaining virus-free insects was the one most often used. It consists in caging virus-carrying insects on rye plants and taking their offspring to other cages before they become adult. In 39 different experiments, colonies of virus-free insects have been obtained in this way from virus-carrying parents. Virus-free colonies were started from each of the 5 different instars of the nymphs. Since this method of obtaining virus-free insects was learned, colonies have been maintained continuously on rye plants. Whenever virus-free insects were required, nymphs were taken from the colonies. The experiments show that wheat and rye, both of

which are favorite host plants for *Cicadula*, are immune to aster yellows. They also prove that the disease is not transmitted through the egg of the carrier insect or by contact of adults with nymphs.

Ten separately caged virus-carrying females were transferred daily for a period of 2 weeks. Several of the plants on which they fed, and in which they deposited eggs, escaped infection. Each plant was kept in a separate insect-proof cage. The colonies that developed on the plants that escaped infection proved to be virus-free.

Twenty colonies of virus-carrying insects were placed on 20 different healthy aster plants in separate insect-proof cages. After 2 days the insects were removed. After 14 days nymphs were present on all the plants. At this time 9 of the plants clearly showed early stages of infection, while the other 11 appeared healthy. The colonies on these 11 plants were removed and caged separately on 11 other young aster plants. Four of the colonies proved to be virus-free; the other 7 were contaminated.

No attempt has been made to obtain virus-free colonies by removing newly hatched nymphs from diseased plants, as was done by Stahl and Carsner (28) in their work with the sugar-beet leafhopper.

#### THE HOST RANGE OF ASTER YELLOWS

The aster yellows disease has been experimentally transmitted by means of *Cicadula sexnotata* to more than 50 different species in 23 different families of plants. It has been taken to one or more species in each of the following families: Compositae, Dipsaceae, Plantaginaceae, Martyniaceae, Gesneriaceae, Scrophulariaceae, Solanaceae, Labiatae, Boraginaceae, Hydrophyllaceae, Polemoniaceae, Asclepiadaceae, Primulaceae, Umbelliferae, Begoniaceae, Resedaceae, Cruciferae, Papaveraceae, Portulacaceae, Caryophyllaceae, Amaranthaceae, Chenopodiaceae, and Polygonaceae. Most of the plants to which the disease has been carried are cultivated flowering plants. It has not yet been transmitted to any crop plant of great economic importance. It should be remembered, however, that aster yellows is identical with a rather serious disease of lettuce known in the West as the Rio Grande disease and here in New York State as the white-heart disease of lettuce. It may also cause a serious disease of buckwheat. Yellows can, no doubt, be transmitted to many species of plants that have not been included in my experiments. There are, however, many immune species. It has not been possible to transmit yellows to any plant in the Leguminosae, Rosaceae, or Gramineae.

None of the symptoms of the disease on aster are shown by all species to which yellows has been transmitted. In certain plants it does not cause the production of secondary shoots, clearing of veins, or upright habit of growth. In a few cases it causes little or no chlorosis. There might be some doubt as to whether the disease was actually carried to plants that do not show the symptoms of yellows as they appear on the aster, if it were not

TABLE I. The Host Range of Aster Yellows

Common Name	Scientific Name	Variety	Family	Yellows Transferred	
				From Aster to New Host	To Aster from New Host
China aster	<i>Callistephus chinensis</i> Nees	Many	Compositae	+	+
Shasta daisy	<i>Chrysanthemum maximum</i> Ramond		"	+	
Paris daisy	<i>C. coronarium</i> L.		"	+	+
Ox-eye daisy	<i>C. frutescens</i> L.		"	+	+
Moon Penny daisy	<i>C. leucanthemum</i> L.		"	+	+
Feverfew	<i>C. leucanthemum maximum</i>	H 1892	"	+	+
Little Gem	<i>C. sp.</i>	H 1864	"	+	+
Feverfew	<i>C. sp.</i>	H 1894	"	+	+
	<i>Matricaria alba</i>	H 2765	"	+	+
	<i>Pyrethrum</i> sp.	H 3552	"	+	+
Swan River daisy	<i>Brachycome iberidifolia</i> Benth.		"	+	+
Ammobium	<i>Ammobium alatum</i> R. Br.		"	+	+
Gaillardia	<i>Gaillardia aristata</i> Pursh		"	+	+
Centaurea	<i>Centaurea margaritae</i> Hort.		"	+	+
Centaurea	<i>C. imperialis</i> Hort.		"	+	+
African daisy	<i>Bellis perennis</i> L.		"	+	+
Salsify	<i>Dimorphotheca aurantiacum</i> DC.		"	+	+
Calendula	<i>Tragopogon porrifolius</i> L.	Manmoth	"	+	+
African marigold	<i>Calendula officinalis</i> L.		"	+	+
Strawflower	<i>Tagetes erecta</i> L.		"	+	+
Lettuce	<i>Helichrysum arenarium</i> DC.		"	+	+
Dandelion	<i>Lactuca sativa</i> L.		"	+	+
	<i>Taraxacum officinale</i> Weber		"	+	+
	<i>Calliopsis</i>		"	+	+
	<i>Cosmidium</i>	H 1602	"	+	+
Daisy fleabane	<i>Erigeron annuus</i> (L.) Pers.	H 1898	"	+	+
Butterweed	<i>E. canadensis</i> L.		"	+	+
Horseweed	<i>Ambrosia trifida</i> L.		"	+	+
Hogweed	<i>A. artemisiifolia</i> L.		"	+	+
Sonchus	<i>Sonchus arvensis</i> L.		"	+	+
	<i>S. oleraceus</i> L.		"	+	+
	<i>Scabiosa atropurpurea</i> L.		"	+	+
	<i>Plantago major</i> L.		"	+	+
	<i>Martynia</i>	H 532	Dipsaceae	+	+
	<i>Gloxinia</i>	H 2300	Plantaginaceae	+	+
			Martyniaceae	+	+
			Gesneriaceae	+	+

TABLE I (continued)

Common Name	Scientific Name	Variety	Family	Yellows Transferred	
				From Aster to New Host	To Aster from New Host
Monkey flower	<i>Mimulus luteus</i> L.	H 3030	Scrophulariaceae	+	+
	Nemesia	H 1580	"	+	
	Calceolaria	H 3675	Solanaceae	+	+
	Schizanthus	H 3602	"	+	
Summer savory	Salpiglossis		Labiatae	+	
	<i>Satureia hortensis</i> L.		"	+	
	Lavender	H 977	Boraginaceae	+	+
	<i>Myosotis scorpioides</i> L.		Hydrophyllaceae	+	+
Cultivated lavender	Nemophila	H 3040	Polemoniaceae	+	
	<i>Phlox drummondii</i> Hook.		"	+	
	<i>P. paniculata</i> L.		Asclepiadaceae	+	
	<i>Asclepias nivea</i> L.		Primulaceae	+	
Forget-me-not	<i>Primula elatior</i> Hill		Umbelliferae	+	
	<i>Didiscus caeruleus</i>	H 2155	"	+	+
	<i>Anethum graveolens</i> L.	H 951	Begoniaceae	+	+
	<i>Pimpinella Anisum</i>		Resedaceae	+	
Dill	<i>Begonia semperflorens</i> Link and Otto		Cruciferae	+	
	<i>Reseda odorata</i> L.		Papaveraceae	+	
	<i>Alyssum compactum procumbens</i>	H 1052	Portulacaceae	+	
	<i>Eschscholzia californica</i> Cham.		"	+	
Anise	<i>Calandrinia grandiflora</i> Lindl.		Caryophyllaceae	+	+
	Portulaca	H 3500	"	+	
	<i>Gypsophila paniculata</i> L.		"	+	
	<i>Silene pendula</i> L.	H 2110	Amaranthaceae	+	
Mignonette	Dianthus		"	+	
	<i>Amaranthus caudatus</i> L.		Chenopodiaceae	+	
	<i>A. aurora</i>	H 1073	Polygonaceae	+	
	<i>Spinacia oleracea</i> L.			+	
Sweet alyssum	<i>Fagopyrum esculentum</i> Moench			+	
				+	
California poppy				+	
				+	
Spinach				+	
				+	
Buckwheat				+	
				+	

Some of the plants to which aster yellows has been transmitted are shown in the table. Each number preceded by the letter H in the column showing the variety indicates that the plant was obtained from seed listed under that number in the 1924 seed catalog of Peter Henderson and Company. The last column shows whether the disease was carried to or from aster from or to each of the new hosts.

for the fact that it was carried from most of the plants back to aster and that the disease so transmitted showed all the symptoms of yellows. Many host plants, however, show symptoms typical of yellows on aster. The most striking symptoms shown by diseased specimens of each of the new host plants listed in table 1 are given below.

*Chrysanthemum maximum* Ramond (Shasta daisy). Plants show general chlorosis and upright habit of growth. Clearing of veins appears in some leaves. Most specimens are only partly diseased.

*C. coronarium* L. Plants are chlorotic and produce many secondary shoots. Petals of ray flowers are much reduced in size and are often a green color.

*C. frutescens* L. (Paris daisy). Plants are dwarfed and chlorotic, as shown in figure A, Plate XLI. Secondary shoots show upright habit of growth. Petals of ray flowers are much reduced in size and are more or less green in color.

*C. leucanthemum* L. (Ox-eye daisy). Plants usually show one-sided infection, chlorosis, and upright habit of growth. Ray flowers are dwarfed and green.

*C. leucanthemum maximum* (Moon Penny daisy, H 1892). Except for slight clearing of veins, the diseased leaves show practically no chlorosis. They are not more erect than normal ones. No extra secondary shoots are produced. This host shows a high degree of tolerance for the disease.

*Chrysanthemum* sp. (Feverfew, H 1864). Plants show chlorosis, upright habit of growth, and many secondary shoots.

*Chrysanthemum* sp. (Little Gem, H 1894). Plants are chlorotic and produce many secondary shoots.

*Matricaria alba* (Feverfew, H 2765). Plants are dwarfed and chlorotic, as shown in figure C, Plate XLI. Many secondary shoots are produced.

*Pyrethrum* sp. (H 3552). Plants are dwarfed and chlorotic. They produce many upright-growing secondary shoots.

*Brachycome iberidifolia* Benth. (Swan River daisy). Plants are dwarfed and chlorotic; secondary shoots are very thin, and leaves are reduced in size.

*Ammobium alatum* R. Br. Plants are dwarfed and chlorotic. They remain in the rosette stage and produce many side shoots. The leaves show bronzing, are turgid and much more brittle than normal ones. They have wavy margins and often turn bottom-side up, as is shown in figure A, Plate XLIII.

*Gaillardia aristata* Pursh. Plants badly dwarfed and chlorotic, with many side shoots, bearing small upright-growing leaves as shown in figure F, Plate XLI.

*Centaurea margaritae* Hort. Plants are stunted and very chlorotic. Numerous side shoots are produced, as shown in figure E, Plate XLI. Stem tissues are chlorotic and show a reddish tinge of color. Some diseased leaves are much longer than normal leaves of the same age.

*C. imperialis* Hort. Plants are stunted and chlorotic, as shown in figure I, Plate XLI. Many upright secondary shoots are produced.

*Bellis perennis* L. Plants are dwarfed and chlorotic. The leaves show a reddish tinge of color along the veins. Leaves are not more erect than those on healthy plants. Secondary shoots die while small.

*Dimorphotheca aurantiacum* DC. (African daisy). Plants are dwarfed and produce many secondary shoots, as shown in figure D, Plate XLI. The leaves and side shoots are very chlorotic. Some of the diseased leaves are very narrow and consist of little more than a midrib. Plants are very chlorotic. The ray flowers are dwarfed and green in color. Many flowers are elongated as they are in the aster. The flower heads are dwarfed.

*Tragopogon porrifolius* L. (Salsify). Plants are dwarfed and chlorotic, as shown in figure G, Plate XLI. Many small secondary shoots are produced. Chlorosis in the leaves of this plant sometimes appears in the form of a pattern composed of areas of tissue of light- and dark-green color.

*Calendula officinalis* L. Plants are dwarfed and chlorotic. The flowers are affected in much the same ways as are those of the aster.

*Tagetes erecta* L. (African marigold). Plants are dwarfed and chlorotic, as shown in figure F, Plate XLII. Secondary shoots develop earlier than in normal plants but are not more numerous. Plants never become bushy, and the habit of growth is not noticeably changed. Plants that are infected when young fail to blossom.

*Helichrysum arenarium* DC. (Strawflower). Plants are dwarfed and chlorotic. They produce many secondary shoots. The flowers are affected very much as are aster flowers.

*Lactuca sativa* L. (Lettuce). Plants are dwarfed and chlorotic. They fail to make heads, but produce many upright secondary shoots. The margins of diseased leaves often show brown-colored pustules which are old latex clots. Flowering side branches are greatly shortened, as shown in figure C, Plate XLIII. Figure F, Plate XLIII, shows the effect of the disease on romaine. The chlorosis of romaine described by Clinton (4) was probably caused by the aster yellows disease.

*Taraxacum officinale* Weber (Dandelion). Plants are chlorotic and produce numerous secondary shoots. The leaves are reduced in width, show clearing of veins and a more upright habit of growth than do the leaves of normal plants. This is shown in figure H, Plate XLI. One of the most striking symptoms on this plant is the bronzing or reddening of the leaves. The flower heads are affected much as they are in the aster.

*Calliopsis* (H 1602). Plants are dwarfed and chlorotic and produce many slender secondary shoots.

*Cosmidium* (H 1898). Plants are dwarfed and chlorotic. Many secondary shoots are produced.

*Erigeron annuus* (L.) Pers. (Daisy fleabane) and *E. canadensis* L. (Butterweed). The symptoms on these two species are essentially alike.

Plants show a great bunching of leaves due to shortening of internodes. They produce large numbers of upright secondary shoots. Leaves are chlorotic and reduced in size. Flowers are affected much as are those of the aster. A diseased and a healthy plant of *E. annuus* are shown in figure B, Plate XLIII.

*Ambrosia trifida* L. (Horseweed): Plants are somewhat dwarfed, as shown in figure E, Plate XLII. Leaves show clearing of veins and are chlorotic while young. Old leaves become a yellowish green color.

*A. artemisiifolia* L. (Hogweed). Plants are stunted and quite chlorotic. Many plants show one-sided infection.

*Sonchus arvensis* L. and *S. oleraceus* L. (Sow thistle). Plants are much stunted and chlorotic. Internodes of stems are shortened, and numerous secondary shoots are produced. Leaves show clearing of veins and are less serrate than normal leaves. Most plants do not flower. A diseased and a healthy plant of *S. oleraceus* are shown in figure A, Plate XLII.

*Scabiosa atropurpurea* L. Plants are stunted and chlorotic. They produce large numbers of secondary shoots.

*Plantago major* L. Plants are chlorotic. Leaves show clearing of veins and upright habit of growth. The petioles are abnormally elongated.

*Martynia* (H 532). Plants are chlorotic and produce numerous secondary shoots.

*Gloxinia* (H 2300). Plants produce many secondary shoots and do not blossom. They are slightly chlorotic.

*Mimulus luteus* L. (Monkey flower). Plants are somewhat dwarfed and slightly chlorotic. The stems are not more than half as thick as those of healthy plants. Many slender secondary shoots are produced. Large numbers of aerial roots grow from the nodes.

*Nemesia* (H 3030). Plants are dwarfed and chlorotic.

*Calceolaria* (H 1580). Plants are chlorotic and much dwarfed. Secondary shoots remain small, and leaves are much reduced in size.

*Schizanthus* (H 3675). Plants are slightly chlorotic with numerous fine secondary shoots, as shown in figure G, Plate XLII. Leaves are much reduced in size.

*Salpiglossis* (H 3602). Plants are much dwarfed and chlorotic.

*Satureia hortensis* L. (Summer savory). Plants are somewhat dwarfed and slightly chlorotic. Numerous secondary shoots show an upright habit of growth.

Lavender (H 977). Plants are stunted and slightly chlorotic. Numerous secondary shoots are produced.

*Myosotis scorpioides* L. Plants are slightly chlorotic and somewhat stunted. They produce numerous upright slender secondary shoots. The leaves are much reduced in size.

*Nemophila* (H 3040). Plants are much dwarfed. The tips of diseased branches are almost white. Numerous short secondary shoots are produced.

Flowers show great variation in size; some are minute. Many of the flowers are of a green color.

*Phlox drummondii* Hook. and *P. paniculata* L. Plants are stunted and chlorotic. The flowers are reduced in size. Many of them are green. Secondary flowers are sometimes produced on the stigmas of other flowers. Such flowers may in turn bear flowers on their stigmas.

*Asclepias nivea* L. Leaves are narrow and yellowish green. They frequently show clearing of veins. A diseased and a healthy plant are shown in figure *E*, Plate XLIII.

*Primula elatior* Hill. Plants are stunted and chlorotic. They produce many secondary shoots. The leaves are greatly reduced in size.

*Didiscus caeruleus* (H 2155). Plants are stunted and chlorotic.

*Anethum graveolens* L. (Dill). Plants are dwarfed and chlorotic, as shown in figure *B*, Plate XLI. They do not produce secondary shoots. Leaves are not more upright in habit of growth than are those of healthy plants.

*Pimpinella Anisum* (Anise, H 951). Plants are stunted and slightly chlorotic. They produce many fine secondary shoots. The leaves are reduced in size. A diseased and a healthy plant are shown in figure *D*, Plate XLII.

*Begonia semperflorens* Link and Otto. Plants are somewhat dwarfed and slightly chlorotic. The leaves are reduced in size and show clearing of veins. Some secondary shoots are produced.

*Reseda odorata* L. (Mignonette). Plants are slightly chlorotic and somewhat dwarfed, as shown in figure *J*, Plate XLI. The most striking symptom is the bronzing and reddening of diseased leaves. The secondary shoots show an upright habit of growth. Secondary flowers are produced on the stigmas of other flowers, as shown in figure *K*, Plate XLI.

*Alyssum compactum procumbens* (Sweet alyssum, H 1052). Plants produce thin chlorotic secondary shoots.

*Radicula palustris* var. *hispida* (Desv.) Robinson. Plants are greatly dwarfed and produce numerous secondary shoots, but show no chlorosis.

*Eschscholtzia californica* Cham. Plants are dwarfed and chlorotic. Many secondary shoots are produced.

*Calandrina grandiflora* Lindl. Plants are stunted and chlorotic. They produce many secondary shoots, as is shown in figure *H*, Plate XLII. A healthy plant is pictured in figure *I*, Plate XLII.

*Portulaca* (H 3500). Plants are dwarfed and chlorotic. Many secondary shoots are produced.

*Gypsophila paniculata* L. Plants are dwarfed and produce many secondary shoots, as shown in figure *B*, Plate XLII. Leaves are chlorotic and show an upright habit of growth. They are often longer than the leaves of healthy plants.

*Silene pendula* L. Plants are stunted and somewhat chlorotic. The leaves are very narrow.



*Dianthus* (H 2110). Plants are stunted and chlorotic.

*Amaranthus caudatus* L. and *A. auroro* (H 1073). Plants are stunted and chlorotic. Numerous short secondary shoots are produced. The leaves show clearing of veins. The disease causes the leaves of *A. auroro*, which are normally red, to become yellowish gray. A diseased and a healthy plant of *A. auroro* are shown in figure F, Plate XLIII.

*Spinacia oleracea* L. (Spinach). Plants are dwarfed and slightly chlorotic. Many upright secondary shoots are sometimes produced. The leaves show clearing of the veins.

*Fagopyrum esculentum* Moench (Buckwheat). Plants are slightly chlorotic and somewhat dwarfed, as shown in figure C, Plate XLII. The diseased plants appear much like healthy ones except that many of the flowers are green and occur in large bunches. An indefinite number of flowers are produced in the leaf axils.

TABLE 2. *The Transfer of Yellows from Various Hosts to Aster*

		Exposed April 24-April 30	Exposed April 30-May 5	Exposed May 5-May 15	Exposed May 15-May 25	Exposed May 25-May 30	No. of Insects Alive May 30
China aster.....	Diseased specimen	+	+	+	+	+	23
	Healthy specimen	+	+	+	+	+	14
Moon Penny daisy.....	Diseased specimen	+	+	+	+	+	20
	Healthy specimen	+	+	+	+	+	13
Little Gem chrysanthemum.....	Diseased specimen	+	+	+	+	+	21
	Healthy specimen	+	+	+	+	+	14
<i>Centaurea imperialis</i> .....	Diseased specimen	+	+	+	+	+	23
	Healthy specimen	+	+	+	+	+	22
<i>Silene pendula</i> .....	Diseased specimen	+	+	+	+	+	10
	Healthy specimen	+	+	+	+	+	1
Calliopsis.....	Diseased specimen	+	+	+	+	+	20
	Healthy specimen	+	+	+	+	+	15
<i>Matricaria alba</i> .....	Diseased specimen	+	+	+	+	+	12
	Healthy specimen	+	+	+	+	+	20
Salpiglossis.....	Diseased specimen	+	+	+	+	+	2
	Healthy specimen	+	+	+	+	+	2
<i>Gaillardia aristata</i> .....	Diseased specimen	+	+	+	+	+	13
	Healthy specimen	+	+	+	+	+	15
Salsify.....	Diseased specimen	+	+	+	+	+	8
	Healthy specimen	+	+	+	+	+	7
Schizanthus.....	Diseased specimen	+	+	+	+	+	2
	Healthy specimen	+	+	+	+	+	1
<i>Amaranthus auroro</i> .....	Diseased specimen	+	+	+	+	+	8
	Healthy specimen	+	+	+	+	+	9
<i>Gypsophila paniculata</i> .....	Diseased specimen	+	+	+	+	+	4
	Healthy specimen	+	+	+	+	+	6
<i>Primula elatior</i> .....	Diseased specimen	+	+	+	+	+	10
	Healthy specimen	+	+	+	+	+	12
<i>Ammobium alatum</i> .....	Diseased specimen	+	+	+	*	+	15
	Healthy specimen	+	+	+	+	+	8

TABLE 2 (continued)

		Exposed April 24-April 30	Exposed April 30-May 5	Exposed May 5-May 15	Exposed May 15-May 25	Exposed May 25-May 30	No. of Insects Alive May 30
<i>Myosotis scorpioides</i> . . . . .	Diseased specimen	+	+	#	#	#	4
	Healthy specimen	+	+	+	+	+	7
<i>Dimorphotheca aurantiaca</i> . . . . .	Diseased specimen	+	+	#	#	+	19
	Healthy specimen	+	+	+	+	+	6
<i>Nemophila</i> . . . . .	Diseased specimen	+	+	#	#	#	9
	Healthy specimen	+	+	+	+	+	10
<i>Mimulus luteus</i> . . . . .	Diseased specimen	+	+	#	#	#	6
	Healthy specimen	+	+	+	+	+	9
<i>Bellis perennis</i> . . . . .	Diseased specimen	+	+	#	#	#	6
	Healthy specimen	+	+	+	+	+	23
<i>Anethum graveolens</i> . . . . .	Diseased specimen	+	+	#	#	#	17
	Healthy specimen	+	+	+	+	+	6
<i>Brachycome iberidifolia</i> . . . . .	Diseased specimen	*	+	+	#	#	14
	Healthy specimen	+	+	+	+	+	10
<i>Chrysanthemum frutescens</i> . . . . .	Diseased specimen	+	+	#	#	#	11
	Healthy specimen	+	+	+	+	*	15
<i>Scabiosa atropurpurea</i> . . . . .	Diseased specimen	#	#	#	#	#	8
	Healthy specimen	+	+	+	+	+	6
<i>Sonchus arvensis</i> . . . . .	Diseased specimen	+	+	#	#	#	14
	Healthy specimen	+	+	+	+	+	14
<i>Erigeron annuus</i> . . . . .	Diseased specimen	+	+	#	#	#	10
	Healthy specimen	+	+	+	+	+	6
<i>Taraxacum officinale</i> . . . . .	Diseased specimen	+	+	#	#	#	20
	Healthy specimen	+	+	+	+	+	16
<i>Ambrosia trifida</i> . . . . .	Diseased specimen	+	+	#	#	#	11
	Healthy specimen	+	+	+	+	+	18

The first column of the table gives the names of the different plants from which yellows was transferred back to asters. A healthy specimen served in each case as a check on the diseased specimen. The dates given at the top of the table show the periods of time during which the five sets of aster plants were exposed. The figures in the last column show the number of insects alive in each culture when the experiment was ended. A plus sign (+) indicates a healthy aster plant; a double plus sign (++) a yellowed plant, and an asterisk (\*) a dead plant.

#### THE TRANSFER OF ASTER YELLOWS FROM VARIOUS HOST PLANTS BACK TO THE ASTER

The transfer of yellows from various host plants to which it was experimentally transmitted back to the aster was described under the section on host range of the disease. One of the experiments in which the disease was taken to aster from several different hosts to which it had been transmitted from aster is given here.

On April 16, 1925, 25 virus-free nymphs in the second, third, and fourth instars were transferred to a yellowed and to a healthy plant respectively of each of the species listed in table 2. The insects were kept on these plants for 8 days, from April 16 to April 24. Each colony was then transferred to a succession of healthy young aster plants. The first set of plants was exposed for 6 days; the second set for 5 days; the third set for 10 days;

the fourth set for 10 days; and the fifth set for 5 days. The results obtained are shown in table 2. A plus sign indicates that the plant remained healthy, a double plus sign that it was diseased, and an asterisk that it died before the transmission record was obtained. The figures given in the last column of the table show the number of insects alive in each colony when the experiment was ended. All check plants remained healthy.

With the exception of the two plants exposed to insects from the diseased aster and the diseased *Scabiosa* plant, all the aster plants of the first set remained healthy. The plant exposed to insects from the yellowed specimen of *Brachycome iberidifolia* died before its transmission record was obtained. All plants of the second set remained healthy except those exposed to insects from the diseased specimens of aster and *Scabiosa*. All plants of the third set exposed to insects from the diseased specimens became diseased except those exposed to insects from *Matricaria alba*, *Gypsophila paniculata*, *Primula elatior*, *Brachycome iberidifolia*, *Amaranthus auroro*, and *Schizanthus*. All plants of the fourth set exposed to insects from diseased specimens became diseased except those exposed to insects from diseased specimens of *Ammobium alatum*, *Primula elatior*, *Amaranthus auroro*, and *Schizanthus*. The plant exposed to insects from *Ammobium* died prematurely. All plants of the fifth set exposed to insects from diseased specimens became diseased except those exposed to insects from *Primula elatior*, *Amaranthus auroro*, and *Schizanthus*. The check plant exposed to insects from the healthy specimen of *Chrysanthemum frutescens* died prematurely.

It will be seen that yellows was transmitted to aster from all the different plants listed in the table except *Amaranthus auroro*, *Primula elatior*, and *Schizanthus*. It is apparently difficult for the insects to obtain the virus from these plants. The colonies of insects from *Schizanthus* were very small, indicating that this plant is not a suitable host for *Cicadula sexnotata*. The insects seem to do well on *Primula elatior*, but it is a plant which they avoid when other suitable host plants are available. *Amaranthus auroro* is, however, a favorite host plant for the insect. The failure of *Cicadula* to transmit yellows from these three plants back to aster is not considered to be evidence that the plants did not have the aster yellows disease. Yellows was repeatedly transmitted to all these hosts. That the disease was transmitted back to aster from the other 25 hosts proves that the symptoms observed on these plants were those of aster yellows. The failure of many of the colonies of insects from diseased specimens to transmit yellows to plants of the first and second sets shows that the incubation period of the virus in the insects of these colonies was not completed during the time the plants were exposed. The plants belonging to the first set exposed to insects from diseased specimens of aster and *Scabiosa* became diseased. This is thought to indicate that the disease is obtained very readily from these plants and that the insects picked up the virus soon after being placed on them. No significant variations were observed in the symptoms or the severity of the

disease carried back to aster from the different host plants shown in the table. Particular attention was given to this point as it was thought that passage through some of these hosts might attenuate the virus and might give rise to weak strains of the yellows disease. No evidence that the virus can be attenuated in this way was obtained in this or in other similar experiments.

#### THE TRANSMISSION OF ASTER YELLOWS THROUGH OLD AND YOUNG LEAVES

Ten adult aster leafhoppers hatched and reared on a yellowed aster plant were confined on a small area of a single old leaf on each of 11 different aster plants. They were confined on the leaves by means of small cages made of glass tubes  $1\frac{1}{2}$  cm. in diameter, open at both ends and fitted into holes in cork stoppers held in place by means of pins stuck into other stoppers placed on the surface of the soil, beneath the leaf to be exposed. The upper end of each cage was covered with cheesecloth. The leaf area to be exposed covered the other end. The cage was held in place by the corks. The insects were confined near the distal ends of the leaves. Five healthy aster plants served as checks. The insects were kept on the leaves for 8 days. They were then removed and the plants were fumigated. Twenty days after the insects were removed, one of the plants showed the first symptoms of yellows. Two days later 4 other plants showed the disease, and still later 4 of the remaining 6 became yellowed. The leaves on which the insects were confined never showed any of the symptoms of yellows. Two of the plants that were exposed as well as the 5 check plants remained healthy. It is interesting to note that 5 of the 9 plants that became diseased showed one-sided infection. In each case of one-sided infection the side of the plant on which the exposed leaf was attached became diseased while the opposite side remained healthy. This suggests that the one-sided infection observed in the field takes place through old leaves.

By means of the same small cages 10 virus-carrying adult aster leafhoppers were confined on a small leaf area near the tip of a single young leaf on each of 6 healthy young aster plants. Two other healthy young plants served as checks. The insects were kept on the leaves for 8 days. They were then removed and the plants were fumigated. Fourteen days after the removal of the insects 3 of the plants showed yellows. Two days later the other 3 plants became diseased. None of these plants showed one-sided infection. The check plants remained healthy.

These experiments prove that yellows can be transmitted through both old and young leaves. They also show that transmission through young leaves produces systemic infection while transmission through old leaves sometimes produces one-sided infection.

## RELATION OF ASTER YELLOWS TO OTHER SIMILAR DISEASES

## The Curly-Top Disease of Beets

The writer's first observations on the symptoms of aster yellows led to the realization that this disease is in many respects similar to the curly-top of beets. It was even suspected that the two diseases might be identical in spite of the fact that curly-top does not cause conspicuous chlorosis. Several experiments were made to test the possibility of these diseases being identical.

On February 4, 1924, 150 adult aster leafhoppers hatched and reared on yellowed aster plants were placed in each of 5 cages containing one healthy aster plant and one each of the following varieties of beet plants: Lanes' Improved White sugar beet, Imperial White sugar beet, Klein Wanzleben sugar beet, and Detroit Dark Red garden beet. One healthy aster plant and one plant of each of the beet varieties were kept free of insects and served as checks. The insects were confined on the plants for 3 weeks, after which time they were removed and the plants were fumigated. All the 5 aster plants exposed to virus-carrying insects became diseased. All beet plants exposed to the same insects and all check plants remained healthy. The experiment proves that *Cicadula sexnotata* is unable to transfer yellows to the varieties of beet plants tested, under conditions favorable for the transfer of yellows to aster plants.

On March 27, 1924, 100 adult virus-free aster leafhoppers were placed on 2 sugar beet plants having curly-top. The diseased sugar beet plants were obtained through the kindness of Mr. Edward A. Schwing of the Spreckels Sugar Company, Spreckels, California. After being confined on the curly-top beet plants for 6 days, they were transferred to a cage containing 2 healthy aster plants and 2 healthy plants of each of the varieties of beets used in the previous experiment. The insects were kept on these plants for 3 weeks. They were then removed and the plants were fumigated. All plants remained healthy during 2 months that they were kept under observation. The experiment proves that under conditions favorable for the transfer of aster yellows the aster leafhopper is unable to transmit curly-top to the varieties of beet plants used or to aster plants.

On October 22, 1925, 154 sugar beet leafhoppers were received from Mr. Eubanks Carsner of Riverside, California. Fifty of the insects obtained in this shipment were placed in a cage containing 2 healthy aster plants and 2 healthy plants of each of the varieties of beets used in the above-described experiments. They were confined on these plants for 21 days. At the end of this time all the beet plants had curly-top disease, but both aster plants were still healthy. The aster plants remained healthy during 2 months that they were kept under observation. The experiment shows that virus-carrying beet leafhoppers do not transmit curly-top to aster plants under conditions favorable for the transfer of this disease to beets.

It was observed that the sugar beet leafhoppers prefer beet plants to aster plants, and it was thought that their failure to transfer curly-top to asters in the above-described experiment might be due to their not feeding sufficiently on these plants. A further experiment was made to test this hypothesis. On November 11, 1925, 10 virus-carrying adult beet leafhoppers were confined on a healthy young aster plant. They were left on the plant until November 30, when it was found that only one of the original 10 insects remained alive. The aster plant was still healthy after 2 months, when the experiment was ended. The experiment proves that the sugar beet leafhopper can live on aster plants. It also shows that this leafhopper is unable to transmit curly-top to asters. Whether the curly-top virus was transmitted to aster without causing any symptoms of disease in this plant is not known, since no attempt was made to transmit the virus from such plants to beet plants by means of virus-free beet leafhoppers.

On January 8, 1926, 10 adult beet leafhoppers that had been confined on a yellowed aster plant for 6 days were transferred to a healthy young aster plant. After 18 days only one of the insects remained alive. On January 26, 10 other beet leafhoppers that had been confined on a yellowed aster plant for 10 days were placed on a healthy young aster plant. After 6 days the insects were removed. Both the aster plants exposed to beet leafhoppers that had fed on yellowed aster plants remained healthy during the 2 months that they were kept under observation. The experiment indicates that the beet leafhopper is unable to transmit the aster yellows disease to aster plants.

The above-outlined experiments bring evidence that *Cicadula sexnotata* can not transmit aster yellows to sugar or garden beets and that it can not transmit the curly-top disease to beets or to aster plants. They also show that *Eutettix tenellus* Baker is unable to transmit the curly-top disease to aster plants under conditions favorable for the transfer of this disease to beets, and that it is unable to transmit aster yellows from diseased to healthy aster plants. This evidence, together with the fact that the aster yellows disease and the curly-top disease differ in their plant host ranges and in certain of the symptoms they produce on their respective hosts, leads to the conclusion that the two diseases, though similar in many respects, are quite distinct.

### The Yellows Disease of the Peach

Peach yellows resembles aster yellows in the stimulation of secondary shoots and in causing these shoots to assume an upright habit of growth. Both cause chlorosis and premature death of diseased plants. Both frequently cause one-sided infection. Peach yellows, however, is not known to cause clearing of veins, a symptom which is characteristic of aster yellows on many different hosts. It also spreads more slowly in the field and has a much more limited geographical distribution than does aster yellows. Aster yellows is a common disease in California and in some other western

states where peach yellows is not known. In spite of these differences it was thought that the two diseases might be identical, *i.e.*, due to the same causative agent.

A number of attempts were therefore made to transfer aster yellows to healthy young seedling peach trees. Since all these attempts resulted in failure, only one experiment will be described in detail in order to illustrate the methods used.

On September 20, 1924, 25 adults of *Cicadula sexnotata*, hatched and reared on yellowed aster plants, were transferred to each of 20 young peach seedlings growing in pots. Fifty similar adults were transferred to each of 8 other healthy young peach seedlings, while 8 additional healthy seedlings of the same age served as checks. After 10 days all the insects confined on these plants were dead. *C. sexnotata* can not live indefinitely on the peach. None of the peach seedlings showed symptoms of yellows during the 2 years they were kept under observation.

Attempts were also made to transfer peach yellows to aster plants by means of *C. sexnotata*. During the summer of 1924, buds from a yellowed Alberta peach tree were budded into healthy young seedlings. In the spring of 1925 the seedlings were cut back and the buds were forced in the usual way. The diseased buds grew into small trees during the summer. Some of these trees brought into a greenhouse in the autumn produced the secondary shoots and yellowed foliage typical of peach yellows. On January 20, 1926, 312 virus-free nymphs of *C. sexnotata* in the first, second, and third instars were confined on one of these yellowed peach trees. After 5 days only 27 of the insects remained alive. They were transferred to a succession of healthy young aster plants as follows: aster number 1 was exposed from Jan. 25 to Feb. 2; aster number 2 from Feb. 2 to Feb. 8; aster number 3 from Feb. 8 to Feb. 13; aster number 4 from Feb. 13 to Feb. 20; aster number 5 from Feb. 20 to Feb. 25; aster number 6 from Feb. 25 to Mar. 1; aster number 7 from Mar. 1 to Mar. 9; aster number 8 from Mar. 9 to Mar. 25; aster number 9 from Mar. 25 to Mar. 28; aster number 10 from Mar. 28 to Mar. 31; and aster number 11 from Mar. 31 to Apr. 5. On March 9, 18 of the original 27 insects remained alive. On April 5, when the last exposure was completed, only one insect was living. All the aster plants remained healthy during the 2 months they were kept under observation. The experiment shows that *Cicadula* can not transmit peach yellows from peach to aster.

Attempts were also made to transfer peach yellows to healthy peach seedlings by means of *C. sexnotata*, but only negative results were obtained.

The experiments described above indicate that *Cicadula* is unable to transfer aster yellows to the peach or peach yellows to peach or to aster. They also bring evidence that aster yellows and peach yellows are distinct diseases. They do not, however, preclude the possibility that some other insect species might be able to transfer aster yellows to the peach.

### The Stunt Disease of Dahlia

Since aster yellows attacks many Composites it was thought that it might go to the Dahlia and that it might be identical with the well known stunt disease of this plant. Attempts were, therefore, made to transmit aster yellows to Dahlia plants by means of *Cicadula sexnotata*. Dahlia seed of the variety Collarette listed in Henderson's seed catalog for 1926 under the number 2072 was planted in flats in a greenhouse. Potted seedlings of this variety were used in all experiments described below.

On March 3, 1926, 100 virus-carrying adults of *Cicadula sexnotata* were placed on each of 2 healthy young Dahlia plants. Two other plants of the same age and variety not exposed to insects served as checks. The insects were confined on the plants for 7 days. They were then removed. The plants were fumigated and placed in an insect-proof cage, where they were kept under observation for 3 months. The 2 plants exposed to insects remained small and produced many secondary shoots, while the check plants grew normally. In order to determine whether the stunted condition of these plants was due to the presence of the aster yellows virus the following experiment was made.

On April 19, 1926, 150 virus-free nymphs of *C. sexnotata* in the third, fourth, and fifth instars were confined on one of the stunted plants exposed to insects in the first experiment. The same number of virus-free nymphs in similar stages of development were confined on one of the check plants. The insects were kept on these plants for one week. Thirty insects from each culture were then placed on each of 4 healthy young aster plants. Thirty adults from a yellowed aster plant placed on another healthy young aster served as a check. These 9 insect cultures were transferred to a succession of healthy young aster plants as follows: the nine plants of the first series were exposed from April 26 to April 28; those of the second series from April 28 to April 30; those of the third series from April 30 to May 4; those of the fourth series from May 4 to May 7; those of the fifth series from May 7 to May 10; and those of the sixth series from May 10 to May 14. The 24 aster plants exposed to insects from the stunted Dahlia plant as well as the 24 aster plants exposed to insects from the normal Dahlia plant remained healthy during 2 months that they were kept under observation. The 6 plants exposed to the culture of insects from the yellowed aster plant all became diseased. The experiment proves that the insects were unable to obtain the aster yellows virus from the stunted Dahlia plant. They did not spread the disease under conditions favorable for its transmission. It also brings evidence that the stunting produced in the first experiment was due to insect injury and not to the aster yellows disease.

In order to test further this conclusion, an experiment in which Dahlia seedlings were exposed to small numbers of virus-carrying and virus-free insects was undertaken. Three insect colonies consisting of 30 virus-carrying adults each and one colony of insects consisting of 30 virus-free



adults were transferred to a succession of healthy young Dahlia plants. The first set of plants were exposed to these colonies from May 21 to May 28; the second set from May 28 to June 1; the third set from June 1 to June 4; the fourth set from June 4 to June 7; the fifth set from June 7 to June 11. Five Dahlia seedlings of the same age and variety served as checks. All plants were kept under observation for 2 months. The plants exposed to virus-carrying insects grew as fast and were as free from secondary shoots as those exposed to virus-free insects. The plants exposed to virus-carrying as well as those exposed to virus-free insects remained normal and similar to the check plants. The experiment proves that when Dahlia seedlings are exposed to only 30 virus-bearing insects for a few days they not only remain free from yellows but suffer no noticeable effects from the feeding of the insects.

The experiments with Dahlia seedlings prove that *Cicadula sexnotata* does not transfer aster yellows to Dahlia plants under conditions favorable for the transmission of the disease. All evidence obtained indicates that the disease known as Dahlia stunt is distinct from aster yellows. Severe injury to Dahlia seedlings caused by the feeding of large numbers of *C. sexnotata* produces stunting and growth of secondary shoots. Such plants resemble those having stunt disease, but it is not considered that stunt disease is in any way related to the feeding of this leafhopper, since *C. sexnotata* does not occur in large numbers on Dahlia plants.

#### Yellows Disease of the Strawberry

In the autumn of 1924, Mr. George M. Darrow of the U. S. Department of Agriculture kindly sent the writer several specimens of yellowed strawberry plants. The plants were placed in a garden near a bed of healthy strawberry plants. During the summer of 1925 it was observed that the yellows spread in some way to previously healthy plants. This yellows disease of the strawberry does not closely resemble aster yellows. Nevertheless, attempts were made to transmit aster yellows to strawberry plants. Thirty virus-carrying adults of *Cicadula sexnotata* hatched and reared on yellowed aster plants were confined on 4 healthy strawberry plants for 5 days. They were then removed and the plants were fumigated. Three other healthy strawberry plants were exposed in a cage containing several hundred virus-carrying leafhoppers for 2 weeks. The plants were then removed and fumigated. Two plants not exposed to insects served as checks. All plants remained healthy during 3 months that they were kept under observation. This experiment brings evidence that aster yellows can not be transmitted to the strawberry by *C. sexnotata* and that it is not identical with the yellows disease observed on the strawberry. Whether this yellows is identical with the strawberry yellows recently described by Plakidas (22) is not known.

### False Blossom of the Cranberry

Some of the symptoms of aster yellows are similar to those associated with the false-blossom disease of the cranberry. It was thought that the two diseases might be identical. Several attempts were made to transmit aster yellows to healthy young cranberry seedlings by means of *Cicadula sexnotata*. Since all these attempts resulted in failure, only one experiment will be described in detail.

On September 20, 1924, 6 healthy cranberry plants were placed in a large cage containing several hundred virus-carrying adults of *C. sexnotata*. They were left in the cage for 2 weeks, after which time they were removed and fumigated. Two similar plants not exposed to insects served as checks. All plants remained healthy during 6 months that they were kept under observation.

Since it was not possible to transmit aster yellows to the cranberry by means of *C. sexnotata*, it is concluded that this disease is distinct from false-blossom.

### FAILURE OF MECHANICAL TRANSMISSION

During the past 3 years many attempts have been made to transmit aster yellows mechanically. The following experiments indicate the methods used.

Portions of yellowed plants were crushed and the undiluted juice was taken for use. The juice was transferred by means of absorbent cotton to wounds made in several different ways. Wounds were made in the tissues of the plants to be inoculated, (1) by jabbing a needle into the lower portions of petioles of the youngest mature leaves; (2) by cutting off the youngest mature leaves; (3) by slitting with scissors the youngest leaves large enough to cut; (4) by tearing with forceps leaves of different ages; (5) by jabbing a needle into stems and buds; and (6) by rubbing and crushing young leaves between thumb and finger. Twelve plants were wounded by each of these methods, and a liberal amount of juice from diseased plants was rubbed into the wounds. Twelve unwounded and uninoculated plants served as checks. The plants were kept in insect-proof cages under favorable conditions for growth. All the plants were from seed planted 46 days before the experiment was started. All plants remained healthy for 2 months after they were inoculated, when the experiment was ended. The experiment shows that yellows is not transmitted under the conditions and by the methods used.

In another experiment, juice from diseased leaves was thoroughly rubbed into young leaves wounded by crushing between thumb and finger. One hundred fifty young plants were subjected to this treatment. All the plants remained healthy during 56 days that they were kept under observation.

Diseased leaf tissue from a yellowed aster plant was crushed and forced into small slits made in the petioles of leaves of 12 healthy young aster plants. Twelve similar unwounded plants kept in the same greenhouse

served as checks. All plants remained healthy during the 2 months the experiment was in progress.

At another time crushed leaf tissues from diseased plants were placed in wounds made in stems and leaf petioles of 25 healthy young aster plants. Juice from diseased leaves was passed through filter paper in order to remove bits of tissue, and was then injected by means of a hypodermic needle into the stems and leaf petioles of 20 other healthy young aster plants. Unfiltered juice from yellowed aster plants was rubbed into wounds made on leaves by scratching lightly with a sharp needle. Six needle scratches were made close together and parallel to the long axis of the leaf, and 6 more at right angles to and across the first 6 scratches. Four leaves were wounded on each plant, and 25 plants were subjected to the treatment. Fresh unfiltered juice was applied to wounds made by rubbing young leaves between thumb and finger. Twenty-five healthy young plants were treated in this way. All the plants used in the experiment were kept under observation for 2 months. All remained healthy.

Four hundred adults of *Cicadula sexnotata* reared on yellowed aster plants were confined in a large test tube for one hour. At the end of this period the bottom and sides of the tube were covered with feces. The insects were removed, and a few cubic centimeters of water were added to the tube. The feces were then dislodged and rubbed up in the water by means of a camel's-hair brush. This mixture was rubbed into 60 needle-jab wounds made in the leaves and petioles of each of 50 healthy young aster plants. The plants were kept under observation for 3 months, but all remained healthy.

Juice was extracted from the crushed leaves of 10 different aster plants that had been diseased for varying periods of time. One of the plants showed the earliest recognizable symptoms of yellows. The other 9 plants had been diseased for periods of time varying from 3 days to 6 weeks. The juice from these plants was mixed and rubbed into 60 needle jabs made in the young leaves of each of 25 rapidly growing, healthy young plants. All the plants remained healthy during 2 months that they were kept under observation.

Two hundred adults of *C. sexnotata* hatched and reared on yellowed aster plants were crushed in a mortar and rubbed up in a small amount of water. The mixture was then rubbed into 60 needle-jab wounds made in the young leaves of each of 25 healthy young plants. All plants used in this experiment were kept under observation for 2 months. All remained healthy.

Buds from diseased branches were budded into the main stems of 12 large, healthy aster plants. The buds were inserted near the ground level. They lived in 7 of the 12 plants. The other 5 buds died. The plants in which the buds grew were cut back to within a few inches of the ground level. This was done in order to force the buds into growth. All the diseased buds produced small diseased branches. The buds in the axils of

leaves above the point where the diseased buds were inserted also grew into short branches. In the cases of 3 of the plants, these branches became yellowed. The other 4 plants died before disease was observed in the branches above the point of insertion of the diseased bud. The experiment shows that yellows can be transmitted by budding. All attempts to transfer aster yellows mechanically, except by budding, have failed.

#### YELLOWS NOT TRANSMITTED THROUGH ASTER SEED

Most virus diseases of plants are not readily transmitted through seeds. Smith (25), working with peach pits from yellowed trees, obtained very poor germination. The pits that germinated gave healthy seedlings. Miss Westerdijk (32) claims that tomato mosaic is transmitted through tomato seed, but Dickson (5) and Gardner and Kendrick (9) grew thousands of plants from seed of mosaic tomatoes without getting a single diseased seedling. Wilbrink and Ledebor (33) and others have shown that sugar cane mosaic is not transmitted by sugar cane seed. Brandes and Klaphaak (2) reached the same conclusion regarding its relation to seeds of corn and of certain wild grasses. According to Doolittle and Walker (6), cucumber mosaic is not transmitted by the seeds of cucumber, squash, muskmelon, and pumpkin, but is transmitted by the seeds of the wild cucumber, *Micrampelis lobata*.

The mosaic diseases of leguminous plants, on the other hand, seem to be quite generally transmitted through seed. This was found to occur in the case of the mosaic of pea bean by Reddick and Stewart (23) and for the mosaic of lima bean by McClintock (16). Gardner and Kendrick (8) have shown that mosaic is transmitted by soybean seed, and Dickson (5) has shown that it is transmitted by the seed of *Trifolium pratense*, *T. hybridum*, *Melilotus alba*, and *Pisum sativum*.

Mention has already been made of the fact that yellowed aster plants often show the symptoms of disease on one side only. The same one-sided infection is also shown by many of the diseased flower heads. Seeds were collected from the diseased and healthy flower heads of a number of aster plants showing one-sided infection. Seeds were also collected from flower heads showing one-sided infection. Seeds from wholly diseased flower heads and from diseased parts of partly diseased flower heads do not germinate. During the fall and winter of 1923 approximately 1,200 aster seedlings were grown from seeds from partly diseased aster plants, and 600 seedlings were grown from seeds from partly diseased flower heads. All these seedlings remained healthy during the 2 months that they were kept under observation. During the 2 years that have passed since these experiments were completed, many hundreds of seedlings have been grown from the seeds of partly diseased aster plants, but in no case have yellowed seedlings been obtained. Chlorotic seedlings occasionally appear but die after a few weeks. They are similar to the albinos produced by many

different species of plants. They do not resemble plants having yellows. It seems safe to conclude that aster yellows is very rarely, if ever, transmitted through aster seeds.

#### OVER-WINTERING OF ASTER YELLOWS

All evidence obtained to date indicates that aster yellows is not transmitted through the seeds of aster plants or through the eggs of the carrier insect. As the aster is an annual and the transmitting insect seems to pass the winter in the egg stage only, these two organisms are probably unable to carry it over winter. It is possible that yellows may be transmitted through the seeds of some host plant other than aster. It is also possible that some other insect may be able to transmit and to carry it through the winter. But biennial and perennial host plants furnish the only means by which aster yellows is now known to overwinter.

In the early spring of 1925 several wild plants of *Plantago major* L., *Chrysanthemum leucanthemum* L., *Sonchus arvensis* L., *Poa annua* L., and a perennial species of *Erigeron* suspected of having yellows, were transplanted together with healthy-appearing specimens of the same species from golf links near the Boyce Thompson Institute to pots placed in insect-proof cages in a greenhouse. On June 20 approximately 100 virus-free nymphs were transferred to a yellowed and to a healthy-appearing plant of *Plantago major*. They were allowed to feed on these plants for 4 days. The nymphs were then removed, and each of the two lots was divided into 4 approximately equal lots of about 25 insects each. They were then separately caged on 8 healthy young aster plants. After 10 days the 8 colonies were transferred to 8 other healthy young aster plants. Six days later they were again transferred to another set of healthy young aster plants on which they were allowed to feed for 6 days. All the plants in the first set remained healthy. Three of the plants in the second set, and the 3 corresponding plants in the third series on which insects from the yellowed plantain had fed, became diseased. The aster plants on which the insects from the healthy plantain fed, and the plants on which one of the colonies from the diseased plantain fed, remained healthy. The plants were then fumigated, and approximately 100 virus-free nymphs were placed on one of the aster plants to which yellows had been transmitted from plantain. An equal number of virus-free nymphs were placed on one of the healthy aster plants. They were kept on these plants for 4 days. The 2 colonies were then transferred to 2 healthy plantain plants. They were left on these plants for 3 weeks. At the end of this period all insects were removed and the plants were fumigated. The plant on which insects from the yellowed aster had fed became diseased; that on which the insects from the healthy aster had fed remained healthy. The diseased plantain showed clearing of veins and other symptoms similar to those observed on the plantain found growing on the golf links. The experiment proves that the disease observed on

*Plantago major* early in the spring is aster yellows and that from such a plant it can be transmitted to asters by *Cicadula sexnotata*.

Ten virus-free adult leafhoppers were placed on yellowed specimens of each of the following plants transplanted from the golf links: *Chrysanthemum leucanthemum* L., *Sonchus arvensis* L., *Poa annua* L., and *Erigeron* sp. The same number of insects were placed on healthy specimens of each of these plants. After feeding on the plants for 6 days all insects were transferred to healthy aster plants on which they were allowed to feed for 3 weeks. The insects from the yellowed *Chrysanthemum*, the yellowed *Sonchus*, and the yellowed *Erigeron* transmitted yellows to the aster plants on which they fed. The insects from the yellowed *Poa annua* plant and those from the 4 check plants failed to transmit the disease. The experiment proves that the yellows found early in the spring on *Chrysanthemum leucanthemum*, *Sonchus arvensis*, and *Erigeron* sp. is aster yellows and that it can be transmitted from these plants to asters by *Cicadula*. It also shows that the yellowed *Poa annua* plants did not have aster yellows. Subsequent experiments in which attempts were made to transfer aster yellows from diseased asters to healthy-appearing *Poa annua* plants proved that this species is not susceptible to the aster disease. The yellowing of the *Poa annua* plants was probably due to some unfavorable soil condition, for when the yellowed plants were placed in rich garden soil they recovered and became normal green in color.

While the above-described experiments prove that the yellows appearing on several wild perennials in June is aster yellows, they do not definitely prove that the disease was carried over winter in these plants. In order to obtain direct evidence on this point, several *Chrysanthemum leucanthemum* plants and one *Plantago major* plant to which yellows was transmitted in the summer of 1925 were planted in a flower garden in the autumn. Healthy check plants of the same species were placed in the same garden. The diseased plants showed yellows on the new growth appearing in the spring of 1926. The experiment proves that these perennials carry the disease over winter. There are doubtless many other susceptible perennials on which the disease may pass the winter. It is thought, however, that the 3 species just mentioned are the most important winter carriers of the disease in the eastern United States.

The Paris daisy and other cultivated chrysanthemums grown in greenhouses also carry yellows over winter. Yellowed Paris daisies were found in several commercial greenhouses during the past two winters. Some of these plants were experimentally proven to have aster yellows. Cuttings made from such plants and put outside during the summer serve as foci of infection. A yellows reported by Nelson (18) on glasshouse-grown chrysanthemums in Michigan is probably identical with the aster yellows disease. *Cicadula sexnotata* is not a greenhouse insect, and so far as known aster yellows never spreads in commercial greenhouses. Many chrysanthemums

are propagated by cuttings which are often placed outside in garden plots during the summer. It is at this time that *Cicadula* transmits yellows to them, but the symptoms of the disease may not be noticeable when the plants are brought into greenhouses in the autumn.

#### INFLUENCE OF YELLOWS ON THE SUSCEPTIBILITY OF PLANTS TO OTHER DISEASES

A species of *Botrytis* which Prof. H. H. Whetzel has kindly informed me belongs to a *Sclerotinia* of the *cinerea* type is a common parasite of the China aster. It causes a damping-off of young plants and a wilt disease of old plants. It frequently forms small black sclerotia on aster seeds. The sclerotia carry the fungus to the seed bed, where numerous conidia are produced. This fungus attacks yellowed plants much more readily than healthy ones. It is the direct cause of the premature dying of most yellowed plants. The yellows disease apparently lowers the resistance of aster tissues to the fungus. It predisposes aster plants to attack by this *Botrytis* in much the same way that sugar cane mosaic predisposes sugar cane leaves to attack by the red-rot fungus *Colletotrichum falcatum* Went (13).

The leaf petioles of several yellowed and healthy aster plants were inoculated with the crown-gall organism, *Bacterium tumefaciens* Smith. Similar galls were produced on both yellowed and healthy plants. The galls on healthy leaves were green while those on yellowed leaves were chlorotic. No difference was observed between the susceptibility of healthy and that of yellowed tissues to this organism.

Aster yellows was readily transmitted to *Asclepias nivea* L., infected with the flagellate *Herpetomonas elmassiani* Migone, by means of *Cicadula sexnotata*. The flagellate flourished in the latex of the yellowed milkweed and seemed unaffected by yellows in spite of the fact that the plant itself showed marked symptoms. The latex of the yellowed plant is apparently as suitable a medium for the growth of the flagellate as is the latex of healthy plants. No noticeable change in the susceptibility of the plant to the flagellate was observed even when the yellows disease had reached an advanced stage.

#### INCUBATION PERIOD OF ASTER YELLOWS IN ITS INSECT CARRIER

During the winter of 1924 several experiments were undertaken to test the ability of the different instars of the nymphs of *Cicadula sexnotata* to transmit aster yellows. Cultures consisting of 100 nymphs in each of the 5 different instars were used. The nymphs were hatched and kept until the time when they were tested on yellowed aster plants. Twenty-five adult insects from similar cultures were used as a check. Each culture was caged separately on a healthy young aster plant for 2 days. The insects were then removed. The plants were fumigated and placed in an insect-proof cage. After 17 days the plant exposed to adult insects became diseased. The other

5 plants that were exposed to nymphs remained healthy during the 2 months that they were kept under observation. The experiment was repeated and again gave the same results.

It was thought that the nymphs might not feed as deeply in the plant tissues as do adults, and that they might be able to transmit the disease if very young plants were used. Nymphs in the fourth and fifth instars were accordingly placed on young aster plants having 2 pairs of leaves only. The tissues of such plants are very tender and easily pierced, but the nymphs failed to transmit the disease. Other experiments proved that adults from nymphs incapable of transmitting yellows can transmit the disease without feeding on yellowed plants after reaching maturity. The nymphs take up the virus. Further experiments showed that virus-free adult insects are unable to transmit yellows directly after feeding on diseased plants. A period of approximately 2 weeks must elapse between the time they feed on such plants and the time when they are first capable of transmitting the virus. This interval is referred to as the incubation period of the virus in the insect. The virus of curly-top of beets has been shown to go through a similar but much shorter incubation period in the beet leafhopper (27, 24). It was found that all instars of the nymphs of *Cicadula* are capable of taking up the virus. They are unable to transmit it because the incubation period in the insect is usually longer than the period necessary for the nymphs to reach maturity.

It was found, however, that under certain circumstances the incubation period is shorter than the time necessary for nymphs to reach maturity, and in that case they are able to transmit the disease. In one experiment 80 newly hatched nymphs were confined on a yellowed aster plant for 14 days. They were then confined for successive periods of time on 3 healthy young aster plants. They were kept on the first plant 4 days, on the second plant 4 days, and on the third plant 7 days. The first plant remained healthy, but the second and third plants became diseased. None of the insects had reached maturity at the end of the 22d day, when they were transferred from the second plant. The virus had, however, completed its incubation period, and the nymphs transmitted the disease. This result was obtained with a culture of insects kept at a relatively low temperature. At temperatures of 70° F. or above most of the insects mature before the incubation period is completed.

The fact that an incubation period is necessary before insects that obtain the virus become inoculative is considered to be important evidence as to the nature of the virus. For this reason the incubation period was carefully studied. Thirty-four experiments were performed which show the existence and approximate length of the incubation period in insects of different ages and with virus obtained from different host plants. The details of some of these experiments are shown in table 3. The numbers at the head of each column in the table indicate the dates on which plants were



exposed to virus-carrying insects. The insects remained on each plant until the next date shown in the table. Plants that remained healthy are indicated by the plus sign; those that became diseased, by the double plus sign. All plants used in the experiments summarized in the table were kept under daily observation. The numbers immediately below each double plus sign show the number of days between the time the plants were exposed to insects and the time when the first symptoms of disease were observed. These figures show in each case the incubation period of the disease in the plant. The first column in the table describes by number the cultures of insects used in each of the 7 experiments. The last column in the table gives, for each experiment, the length of the incubation period of the virus in the insects used. Vigorous colonies of insects were used in every case. Before some of the experiments were ended, however, the colonies had grown old. All plants were kept under observation until May 15.

The insect culture described as number 1 in the table consisted of 30 virus-free nymphs in the first, second, and third instars. The number in each instar was not recorded. The colony was placed on a yellowed *Calendula* plant on January 20. On January 25 it was removed and placed on the first plant shown in the table. The insects remained on this plant until January 28. From this date until March 13 they were transferred daily to new plants. They were then transferred to new plants every 2 days or after longer intervals as shown in the table. The culture was transferred at frequent intervals until April 15, when the experiment was ended, but only the results obtained to March 22 are shown in the table. The 10 plants on which the insects fed from January 25 to February 5 remained healthy. Of the 47 plants on which they fed from February 6 to April 15 all but 3 took the disease. The 3 plants that escaped infection were exposed after March 28, when the insect culture was becoming old and weak. Only 2 of the original 30 insects were alive on this date. The experiment shows an incubation period in the insect of from 12 to 17 days: 17 days if the virus was picked up on the first day the insects were on the yellowed *Calendula* plant, and 12 days if it was picked up on the last day.

The insect culture described in the table as number 2 consisted of 30 nymphs in the first instar when the experiment was started on January 15. The insects were hatched and kept on a yellowed aster plant until taken for use in the experiment. They varied in age from a few hours to 3 days. None was more than 3 days old. They were kept from January 15 to January 28 on 4 healthy young aster plants. The plants were exposed for various periods of time as shown in the table. All remained healthy. From January 28 to March 13 they were transferred daily to new plants. All plants exposed to the culture from January 29 to March 3 became diseased. All plants exposed after March 3 remained healthy. Twelve of the original 30 insects were living on this date. It is possible that these 12 insects were the youngest in the culture and that they were taken from the



diseased plant on which they hatched before they had opportunity to feed. An incubation period of the virus in the insects of from 14 to 17 days is shown by this experiment.

The insect culture described as number 3 in the table consisted of 100 virus-free adults when the experiment was started January 28. The insects were placed on a yellowed aster plant on January 28 and were removed on January 29. The first 36 plants used in this experiment are shown in Plate XL. The insect colony was kept on each plant for one day. The plant shown on the left side of the picture was diseased long before the experiment was started, and is the one from which the insects obtained the virus. They were kept on the plant exactly 24 hours. From January 29 to March 13 they were transferred daily to new plants. The 11 plants on which the insects fed from January 29 to February 8 remained healthy. The 21 plants on which they were cultured from February 9 to March 1 became diseased. The next 2 plants remained healthy, while the last of the 36 plants shown in the picture became diseased. The plants exposed on March 4, 7, 8, and 9 became diseased. The other plants exposed after March 1 remained healthy. Thirteen of the original 100 insects were living on that date. It is possible that most of these insects did not obtain the virus during the 24 hours they were on the diseased plant. The experiment shows an incubation period of 12 days.

The insect culture described as number 4 in the table consisted of 100 virus-free adults when the experiment was started. They were allowed to feed on a yellowed aster plant from January 26 to January 29. After this date they were transferred daily to new healthy young aster plants until March 7. All the 14 plants exposed from January 29 to February 11 remained healthy. All the 24 plants exposed between February 12 and March 7 became diseased except that exposed March 5, when the culture was old and weak. On that date, 16 of the original 100 insects were living. It is possible that these insects failed to obtain the virus during the 3 days they were on the yellowed aster plant. An incubation period in the insects of from 14 to 17 days is shown in this experiment; 17 days if the virus was picked up on the first day the insects were on the yellowed plant, and 14 days if it was picked up on the last day.

The insect culture described as number 5 in the table consisted of 30 virus-free nymphs in the first instar when the experiment was started. They were confined on a yellowed *Calendula* plant from January 13 to January 15. They were then transferred to new plants at intervals, as shown in the table. From January 28 to February 17 they were transferred daily. The 3 plants on which the insects fed from January 15 to January 27 remained healthy. The 21 plants on which they fed from January 28 to February 17 became diseased. An incubation period in the insects of from 13 to 15 days is shown in this experiment; 15 days, if the virus was picked up on the first day the insects were on the yellowed *Calendula* plant, and 13 days if it was picked up on the last day.

The insect culture described as number 6 in the table consisted of 30 virus-free adults when the experiment was started. They were confined on a yellowed aster plant for 2 days, from January 26 to January 28. From January 28 to February 17 they were transferred daily to new healthy young aster plants. All the plants on which they fed from January 28 to February 6 remained healthy. All the plants on which they fed after February 6 became diseased. An incubation period of from 10 to 12 days is shown in this experiment; 12 days if the virus was picked up on the first day the insects were on the yellowed aster plant, and 10 days if it was picked up on the last day.

The insect culture described as number 7 in the table consisted of 30 nymphs in the first, second, and third instars when the experiment was started. No record was made of the numbers in each instar. The insects were placed on a yellowed *Calendula* plant on January 20 and were kept on the plant until January 25. On the latter date they were placed on a healthy young aster plant. They were kept on this plant for 3 days, after which they were transferred daily until February 17. The twelve plants on which they fed from January 25 to February 7 remained healthy. The 10 plants on which they fed from February 8 to February 17 became diseased. The experiment shows an incubation period in the insect of from 14 to 19 days.

The results obtained with the different insect colonies described in the table are in close agreement, although some variation is shown in the length of the incubation period in the different colonies. All the experiments show that virus-free insects are unable to transmit yellows immediately after feeding on yellowed plants. An interval of at least 10 days was necessary in every case before any of the colonies were inoculative. With one exception, 10 days is the minimum incubation period shown by the virus in the 34 insect colonies that have been tested. In one experiment a plant, exposed 6 days after the insects used had first fed on a yellowed plant, became diseased. Six other plants following this one in the series, and exposed to the insects after this plant, remained healthy. It is thought that the plant had been accidentally inoculated before being used in the experiment. If this assumption is correct, the minimum incubation period is not less than 10 days. It is interesting to note that the minimum incubation period of the virus in the insect is of approximately the same length as the minimum incubation period of the disease in aster plants.

A study of the table will show that the incubation period of the disease in different aster plants varies between rather wide limits. The average incubation period in the plants used in the experiments summarized in the table is about 18 days. The shortest period shown is by a plant used in experiment number 1 and exposed February 19. The longest periods shown are by plants exposed on February 6 and February 12 in experiments numbers 1 and 4. The plant in experiment number 1 gave an incubation period of 39 days; that in experiment number 4, one of 38 days. This

period is considerably longer than that shown by any of the other plants and is more than twice the average incubation period shown by the other plants. It is interesting that the disease was transmitted to both these plants by insects in which the virus had just finished its incubation period. This suggests that the changes which probably occur during the incubation period of the virus in the insects may not have been completed when the virus was transmitted to the plants, but that they had progressed sufficiently to make possible a slow development of the disease in the plants. That the condition of the virus in the insect can influence the length of the incubation period in the plant is evidence in favor of the view that the causative agent is biological rather than chemical.

#### RETENTION OF THE VIRUS BY INSECTS CULTURED ON IMMUNE PLANTS

Many attempts were made to determine whether colonies of insects confined on plants immune to yellows would lose the virus.

On December 4, 1925, 30 nymphs in the first instar, which had been hatched and kept on a yellowed plant, were transferred to rye plants. They were kept on these plants for 33 days. The rye plants remained healthy in appearance. The 25 insects that were alive at the end of this period were transferred to a healthy young aster plant. After 6 days they were removed and placed on a second aster plant. After 11 days they were placed on a third, and after 7 days on a fourth, aster plant. They were kept on this last plant for one week. All the aster plants became diseased. The rye plants on which the cultures had been kept for 33 days were fumigated and placed in another cage. Thirty virus-free nymphs were confined on these plants for one month. They were then transferred to a succession of 4 healthy aster plants. They were kept on each of these plants for one week. The four plants remained healthy during the 2 months they were kept under observation. The experiment proves that nymphs in the first instar take up the virus and are able to retain it for at least 33 days when kept on rye plants. It also shows that rye plants do not carry the virus.

On November 18, 1925, 30 nymphs in the first instar, hatched and kept on a yellowed aster plant, were transferred to rye plants. They were kept on these plants for a period of 7 weeks. The colony was then transferred successively to 4 different aster plants. It was kept on the first plant 6 days, on the second, 11 days, on the third, 7 days, and on the fourth, 8 days. All the aster plants became diseased. The rye plants were fumigated and placed in an insect-proof cage. Thirty virus-free nymphs in different instars were confined on these plants for 2 weeks. They were then transferred successively to 4 healthy aster plants. They were kept on these plants for periods of 6, 11, 7, and 8 days. All 4 plants remained healthy during 2 months that they were kept under observation. The experiment proves that the virus is retained by the insects for at least 49 days.

On January 19, 1926, 100 nymphs in the first instar, hatched and kept on a yellowed aster plant, were transferred to rye plants. The colony was kept on these plants for 2 months. On March 19, the insects were taken from the rye plants and transferred successively at intervals of a few days to 8 different healthy young aster plants. The transfers were made on the following dates: March 19, 22, 25, 27, 29, 31, April 1, and April 5. All 8 aster plants exposed to these insects became diseased. The rye plants were fumigated, and 30 virus-free nymphs were confined on them for 2 weeks. The colony was then removed and transferred successively to 4 different aster plants. The insects were confined on each of these plants for 7 days. All remained healthy. The experiment proves that the virus was retained by the insects during the 59 days they were on the rye plants and during an additional 16 days that they were kept on healthy aster plants. Only 7 of the original 100 insects were alive when the experiment was ended. They retained the virus for a period of 75 days. During this time the insects passed through the 5 instars to maturity and old age. The fact that they retain the virus over such a long period of time is considered to be evidence in favor of the view that the causative agent is biological rather than chemical.

#### INSECT EXPOSURE TO YELLOWED ASTER PLANTS

It was observed in early experiments that certainty of transmission of aster yellows by cultures of *Cicadula sexnotata* depends on the number of insects used and on the length of time they are kept on yellowed plants. Many insect cultures readily transmit yellows when young but fail to transmit it when they grow old. Old colonies of insects are always reduced in numbers. It was thought that virus-carrying individuals might be shorter-lived than virus-free individuals, and that, when cultures grow old, only virus-free insects are left.

Several experiments were made to determine the exposure necessary for a medium-sized insect culture to obtain the yellows virus. Twenty virus-free adults were confined for 2 hours on each of 3 yellowed aster plants. Twenty similar adults confined for the same period of time on a healthy plant served as a check culture. Each culture was then transferred to a succession of 4 healthy aster plants. The colonies were kept on each plant for one week. All plants remained healthy, showing that a feeding period of 2 hours is not long enough to render colonies virus-bearing.

In another experiment, 25 virus-free adults were confined on a yellowed aster plant for one day. Twenty-five similar adults kept for the same length of time on a healthy aster plant served as a check culture. Both cultures were transferred to a succession of 4 healthy aster plants. They were confined on each plant for one week. The last 2 plants on which the insects exposed to the diseased aster fed became diseased. All other plants remained healthy. The experiment shows that an exposure of one day is

sufficient to enable the insects to become virus-bearing. The number of insects that obtain the virus in that time is not shown by this experiment.

It was suggested that insects might be able to take the virus from young leaves only, and that those insects that happened to feed on old leaves during their exposure to yellowed plants might not become disease carriers. An experiment was undertaken to test this hypothesis. Fifty virus-free nymphs in the first instar were placed in a large test tube containing old aster leaves from a yellowed plant. Thirty virus-free adults were placed on similar leaves in another tube. Fifty virus-free nymphs and 30 virus-free adults were placed in 2 other tubes containing young leaves from yellowed aster plants. Fifty similar nymphs and 30 virus-free adults placed in 2 other tubes containing both old and young leaves from healthy aster plants served as check cultures. All insects were kept in the tubes for 2 days. Each culture was then transferred to a succession of 4 healthy young aster plants. The insects were kept on the first set of plants for 10 days; on the second set for 5 days; on the third set for 6 days; and on the fourth set for 2 days. The last 2 plants on which the insect cultures exposed to diseased leaves fed became diseased in every case. All other plants remained healthy. The experiment proves that both nymphs and adults are able to obtain the virus from both old and young leaves.

It was also thought possible that certain individual insects might be immune to the virus and might be able to feed on yellowed plants indefinitely without becoming carriers of the disease. An experiment was, therefore, undertaken in which individual insects were employed for yellows-transmission. All the insects used in this experiment were from eggs laid in yellowed aster plants, February 19, 1926. The plants were kept in a greenhouse held at temperatures between 70° and 75° F. By March 24 many of the insects hatched from these eggs were adults. Since, at the temperatures at which the eggs were held, it takes about 12 days for them to hatch, it may be assumed that most of the insects were about 3 weeks old on this date. One of these young adults was placed in each of 30 lantern-globe cages. Thirty similar adults were placed in a single cage. The colony of 30 insects was designated as culture *A*. The other 30 insects were given culture numbers from 1 to 30. A succession of 23 healthy young aster plants were exposed to the colony and to each of the individual insects that lived until the experiment was ended. The different sets of plants were exposed for varying periods of time. None was exposed for less than one day and none for more than 7 days. In this way the transmission record of the colony and of each of the 30 individual insects has been obtained. The exposures were made on the dates and for the periods of time shown in table 4. All plants were kept under observation for at least 6 weeks after the insects were removed. The results are shown in the table. The plus sign indicates that the plant remained healthy; the double plus sign that it became diseased; and the asterisk that it died before the incubation period

TABLE 4. *Individual Transmission Records*

Culture number.....	A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Exposed March 24-March 30.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" March 30-April 5.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 5-April 10.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 10-April 16.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 16-April 21.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 21-April 28.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 28-April 29.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 29-April 30.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" April 30-May 1.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 1-May 3.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 3-May 4.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 4-May 5.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 5-May 7.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 7-May 10.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 10-May 14.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 14-May 21.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 21-May 28.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" May 28-June 4.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" June 4-June 11.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" June 11-June 18.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" June 18-June 25.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" June 25-July 2.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
" July 2-July 9.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Transmission records of 30 individual insects and of a colony consisting of 30 insects. A plus sign (+) indicates a healthy plant; a double plus sign (++) a yellowed plant; an asterisk (\*) a dead plant. A minus sign (-) indicates the death of the insect used. The dates given in the first column show the periods during which each set of plants was exposed.



for the disease in the plant was ended. The minus sign indicates that the insect was dead on the date of beginning the exposure under which the sign appears.

It will be seen that the incubation period of the virus in most of the insects had not been completed on March 30, when the first exposure was ended. Only the colony and insects numbers 4 and 6 transmitted the disease. During the exposure of the second set of plants the colony and insects numbers 1, 2, 4, 6, 8, 11, 13, 14, 20, 24, and 30 transmitted the disease. During the exposure of the third set of plants the colony and all the insects except numbers 2, 3, 9, 15, 16, 18, 19, 21, 23, 26, 27, and 28 transmitted yellows. Insect number 26 died during the exposure of the third plant. During the exposure of the fourth set of plants all the insects except numbers 3 and 28 transmitted the virus. In the fifth exposure all transmitted it except number 3. Numbers 3, 17, and 25 died during this exposure. The sixth set of plants was exposed for 7 days. The plant on which insect number 24 fed died prematurely. The disease was transmitted to all other plants except the 2 on which insects numbers 29 and 30 were confined. Insect number 14 died during the exposure. The seventh set of plants was exposed for only one day. During this period all insects transmitted the virus except numbers 5, 6, 18, 27, and 30. Insect number 27 died during this exposure. The eighth set of plants was also exposed for only one day. All insects transmitted the virus except numbers 5, 6, 18, 22, 23, and 28. The ninth set of plants was exposed for one day. All insects transmitted the virus except numbers 5, 6, 11, 18, 22, 23, 29, and 30. The tenth set of plants was exposed for two days. The virus was transmitted by all insects except numbers 5, 6, 18, 19, 22, 23, and 30. Insect number 2 died during the exposure. The eleventh set of plants was exposed for one day. The disease was transmitted by all insects except numbers 4, 5, 6, 7, 8, 10, 18, 22, 23, 28, and 29. The twelfth set of plants was exposed for one day, and the virus was transmitted by all insects except numbers 5, 6, 7, 10, 18, 19, 22, 23, 28, and 29. The plant exposed to insect number 19 died prematurely. The thirteenth set of plants was exposed for two days. All insects transmitted the virus except numbers 5, 6, 7, 8, 18, 22, 28, and 30. The plants on which numbers 5 and 6 fed died prematurely. Insect number 30 died during the exposure. The fourteenth set of plants was exposed for 3 days. The plant on which insect number 5 fed died before the transmission record was obtained. The virus was transmitted by all other insects except numbers 6, 8, 18, and 22. The fifteenth set of plants was exposed for 4 days. The plant on which insect number 5 was confined died prematurely. The disease was transmitted to the plants on which all other insects fed except numbers 6, 7, 8, 10, 18, 22, 23, 24, and 28. Insect number 6 died during this exposure. The sixteenth set of plants was exposed for 7 days. The plants on which insects numbers 18 and 20 fed died prematurely. All insects transmitted the disease except numbers 5, 8, 18, 20, and 22. Insects numbers

1 and 20 died during the exposure. The seventeenth set of plants was exposed for 7 days. All insects transmitted the virus except numbers 18, 19, and 22. Insect number 19 died during the exposure. The eighteenth set of plants was exposed for 7 days. The plant on which insect number 22 fed died before the transmission record was obtained. All other insects transmitted the virus except numbers 10, 11, and 18. The nineteenth set of plants was exposed for 7 days. All insects transmitted the virus except numbers 7, 10, 11, 18, 22 and 23. The twentieth set of plants was exposed for 7 days. All insects transmitted the disease except numbers 10, 18, 22, and 24. The twenty-first set of plants was exposed for 7 days. The plant on which insect number 12 fed died prematurely. Insects numbers 7 and 10 died during the exposure. All other insects transmitted the virus except numbers 7, 8, 10, 18, 22, and 23. The twenty-second set of plants was exposed for 7 days. All insects transmitted the disease except numbers 12, 18, 22, and 23. The one insect that remained alive in culture *A* failed to transmit the virus. It died during this exposure. Insect number 29 also died. The twenty-third set of plants was exposed for 7 days. All insects transmitted the virus except numbers 18, 22, and 23. On July 9, when the experiment was ended, only 6 of the insects were living.

The table shows the transmission record of culture *A* and of the several individual insect cultures used. Yellows was transmitted to all plants exposed to culture *A* except the last. The single insect that was living when this plant was exposed died during the exposure. The record shows that the culture was inoculative for at least 80 days, from March 30 to June 18. Yellows was transmitted to all except the first plant exposed to insect number 1. During the time of this exposure the virus had doubtless not completed its incubation period in the insect. This leafhopper died during the exposure of the sixteenth plant. It, nevertheless, transmitted yellows to this plant and to all the other 15 plants on which it fed after the termination of the incubation period of the virus. The insect was inoculative for at least 39 days, from April 5 to May 14. Insect number 2 was not inoculative during the time the first plant was exposed. It transmitted yellows to the plant exposed from March 30 to April 5, but failed to transmit it to the plant on which it fed from April 5 to April 10. The virus was carried to all other plants on which it was confined. The insect died during the exposure of the tenth plant, but transmitted yellows to this plant. It was inoculative during a period of at least 26 days, from April 5 to May 1. Why it did not transmit yellows to the plant on which it fed from April 5 to April 10 is not known. Insect number 3 died during the exposure of the fifth set of plants. It failed to transmit the disease to any of the plants on which it fed. This does not prove that it did not obtain the virus. The insect died during the exposure of the fifth plant, and may not have fed on this plant. Insect number 28 also failed to transmit yellows to the first 4 plants on which it was confined. It is assumed that the incubation period of the virus in insects

numbers 3 and 28 was not completed during the exposure of the first 4 sets of plants. Insect number 4 transmitted yellows to all but one of the 20 plants on which it was confined. This plant was exposed for only one day. It was inoculative for a period of at least 73 days, from March 30 to June 11. Insect number 5 failed to transmit yellows to the first 2 plants on which it fed. The incubation period of the virus was probably not completed during the time these plants were exposed. It transmitted the disease to the next 4 plants, but failed to transmit it to the 10 plants on which it fed from April 28 to May 21. Three of these plants died prematurely. It was apparently unable to transmit yellows to plants on which it was confined for no longer than one or two days, and even failed to transmit it to plant number 16 on which it was confined for one week. It did, however, transmit the virus to all the 4 plants on which it fed during the last 4 weeks of its life. Insect number 6 transmitted the virus to the first 6 plants on which it was confined. It failed to transmit it to any of the plants exposed after April 28. Insect number 7 failed to transmit yellows to the first 2 plants, transmitted it to the next 8 plants, failed to transmit it to the next 3, transmitted it to the fourteenth plant, failed to transmit it to the fifteenth plant, transmitted it to the sixteenth, seventeenth, and eighteenth plants, failed to transmit it to the nineteenth plant, transmitted it to the twentieth plant, and failed to transmit it to the twenty-first plant. This insect was inoculative over a period of at least 62 days, but for some reason did not transmit the disease to many of the plants on which it was confined. Insect number 8 also transmitted yellows over a long period of time, but without taking it to all plants exposed. It was inoculative for at least 88 days, from April 5 to July 2. After the completion of the incubation period it carried the virus to all but 6 of the 22 plants on which it fed. Insect number 9 failed to transmit the virus to the first 3 plants on which it was confined. The incubation period had undoubtedly not been completed during the time these plants were exposed. After this period was terminated the insect transmitted yellows to all the 20 plants on which it was confined. It was inoculative for a period of at least 77 days. Insect number 10 failed to transmit yellows to the first 2 plants on which it was confined, but carried it to 12 of the 19 plants on which it fed during the remainder of its life. Insect number 11 failed to take yellows to the first plant but transmitted it to all but one of the next 16 plants. The 2 plants on which it was confined shortly before its death remained healthy. Insect number 12 failed to transmit yellows to the first 2 plants. It carried the disease to all but 2 of the other 21 plants. One of these plants died prematurely; the other remained healthy. This insect was capable of inoculating plants over a period of at least 83 days. Insect number 13 failed to transmit yellows to the first plant on which it was confined, but carried it to all the other 17 plants on which it fed until the time of its death. Insect number 14 carried yellows to all except the first plant. It died prematurely. Insect number 15 transmitted

the disease to all except the first 3 plants. It was inoculative over a period of at least 56 days. The transmission record of insect number 16 is exactly like that of insect number 15, except that number 15 lived a few days longer and transmitted yellows to one more plant than did number 16. Insect number 17 died prematurely. It failed to transmit yellows to the first 2 plants on which it was confined but carried it to 3 other plants. Insect number 18 failed to transmit yellows to the first 3 plants but took it to the next 3. It failed to transmit the disease to any of the plants on which it fed thereafter. Insect number 19 also failed to transmit yellows to the first 3 plants. It carried the disease to 11 of the other 14 plants on which it fed. One of the 3 healthy plants died prematurely. The other 2 remained healthy. Insect number 20 transmitted yellows to all the plants on which it was confined except the first and last. The last plant died before the transmission record was obtained. The insect was inoculative for at least 35 days. Insect number 21 transmitted the virus to all plants on which it was confined except the first 3. It was inoculative for at least 49 days. Insect number 22 failed to transmit yellows to the first 2 plants but carried it to all the next 5. It failed to transmit the disease to any of the 16 plants on which it fed thereafter. This insect was inoculative for not more than 19 days. Insect number 23 failed to transmit yellows to the first 3 plants but did transmit it to the next 4. It carried the disease to only 6 of the next 16 plants on which it was confined. Insect number 24 transmitted yellows to all plants on which it was confined except the first, sixth, fifteenth, and twentieth. No record was obtained for the sixth plant because of its early death. The insect died during the exposure of the twentieth plant. Insect number 25 died during the exposure of the fifth plant. It transmitted yellows to all except the first 2 plants. Insect number 26 died during the exposure of the third plant. It failed to transmit yellows to the plants on which it fed. The virus had probably not completed its incubation period when the insect died. Insect number 27 failed to transmit yellows to the first 3 plants, but carried it to the fourth, fifth, and sixth plants. It died during the exposure of the seventh plant, to which it failed to transmit the disease. Insect number 28 failed to transmit yellows to the first 4 plants but carried it to the next 3 and to all but 5 of the 11 plants on which it was confined during the remainder of its life. Insect number 29 failed to take yellows to the first 2 plants but carried it to all but 4 of the remaining 20 plants. Insect number 30 failed to transmit yellows to the first plant on which it fed, but did transmit it to all but 5 of the other 12 plants exposed.

These 30 transmission records show that all insects that lived long enough to give a transmission record were virus-carriers. Both males and females were included in the group of individuals tested. Insects numbers 4, 13, and 15 were females. Insects numbers 5 and 28 were males. Considerable variation is shown in the certainty with which different individuals

transmit the disease. Ten insects transmitted it to every plant on which they were confined after the completion of the incubation period. Three of these, numbers 14, 17, and 25, were short-lived. The other 7, consisting of numbers 1, 9, 13, 15, 16, 20, and 21, transmitted yellows over a long period of time and with great certainty. A few other insects, like numbers 2, 4, and 12, transmitted it to almost all the plants on which they fed and should probably be placed in this class. Another group of insects, of which numbers 5, 7, 8, 10, 23, 28, 29, and 30 are examples, transmitted yellows over a long period of time but with considerable irregularity. Many of the plants on which they were confined escaped the disease. Still another group transmitted yellows to a few plants and then apparently lost the virus. Insect number 6 transmitted the disease to the first 6 plants on which it fed but failed to transmit it to any of the 8 plants on which it was later confined. After the completion of the incubation period of the virus, insect number 18 transmitted yellows to 3 plants. It was kept on the first, second, and third plants for periods of 6, 5, and 7 days respectively. It failed to transmit yellows to any of the 16 plants on which it was confined during the following 72 days. Number 22 is another example of an insect that lost its ability to transmit yellows. It carried the disease to the first 5 plants on which it fed after the completion of the incubation period. It failed to transmit it to any of the 15 plants on which it was confined during the following 71 days.

It is interesting to note that 6 of the 30 insects lived during the 107 days this experiment was in progress. They were all from eggs deposited 33 days before the experiment was started. If the eggs hatched in 12 days, these insects were 128 days old when the experiment was ended. When *Cicadula sexnotata* is confined in large colonies, its average length of life is not more than 60 days. All but one of the insects in culture A died before May 28. Eighteen of the 30 insects kept in individual cages were alive on this date. *Cicadula* lives much longer in individual cages than it does when grown in colonies, even though the colonies are small and are given an abundant food supply. Three of the insects alive when the experiment was ended transmitted yellows to the last plants on which they were confined, showing that they carried the virus for more than 100 days. The other 3 insects were not inoculative during the last 3 weeks of the experiment.

This experiment brings evidence that most, if not all, individuals of *C. sexnotata* are capable of taking up the aster yellows virus. Some transmit the disease to all plants on which they feed for as long as one day, while others transmit it with much less certainty. Many individuals carry the virus as long as they live. Some, however, appear to lose it after a short time.

#### EFFECT OF YELLOWS ON ITS INSECT CARRIER

When it was found that a specific relationship exists between aster yellows and its insect carrier, an effort was made to determine whether yellows has any observable effect on its insect host. Cultures of virus-carrying and virus-free leafhoppers were kept under identical conditions

of light, temperature, and humidity on similar host plants in cages in a greenhouse. It was thought that the virus-carrying insects might be shorter-lived than those that are virus-free. It was found, however, that the average length of life of virus-bearing insects is approximately the same as that of virus-free insects. Since the virus causes chlorosis in green plant tissues and partial or complete loss of pigment in colored flowers, it was thought that it might affect in some way the color of the virus-carrying insects. Such insects in various stages of development were carefully compared with virus-free insects in corresponding stages of development. No differences in color could be observed between the two kinds of insects. They were also compared for differences in size and tested for resistance to heat and desiccation, but no differences whatever could be noted. Virus-free and virus-bearing nymphs and adults were fixed in Carnoy's fixative, imbedded in paraffin in the usual way, sectioned with a microtome, and stained with Flemming's triple stain. This method of fixing and staining gave good preparations, but no differences could be found in the morphology or structure of internal organs or in the reactions of cells in the several tissues to the stains. There are certainly no conspicuous differences between insects that bear the virus and those that do not.

#### DISCUSSION

Aster yellows is an infectious chlorosis of the China aster prevalent in North America but not known in other parts of the world. Its characteristics place it in the group of virus diseases, but it has not been shown to be due to a filterable virus. It is transmitted by a leafhopper which was probably imported into the United States 50 or more years ago. This insect is common in Europe and the Orient, where the aster and other host plants of the disease are extensively grown. Aster yellows, however, does not occur in European and Oriental countries apparently because the virus of this disease has not yet reached them.

The aster yellows disease is doubtless endemic in North America. It probably occurred unnoticed on some wild host plant long before the European leafhopper *Cicadula sexnotata* and the Oriental aster *Callistephus chinensis* were brought to the United States. It may have been and may still be transmitted by some insect endemic in the United States. The disease was probably of little importance, however, until after the importation of *Cicadula sexnotata*. This insect, being very active and living and breeding on many different species of plants, makes aster yellows a serious disease and its control an important problem for those who grow the China aster or other cultivated host plants of the disease.

The most promising means of control that can be suggested at present are eradication of weed hosts in the vicinity of aster plantings, destruction of all aster plants as soon as they are observed to show yellows, and spraying or dusting aster plots with nicotine or other suitable sprays or dusts. Asters grown in plots surrounded by cultivated fields are less subject to severe

infection than asters grown in the vicinity of pastures, meadows, waste lands, or other weedy places. Aster beds near buildings are somewhat less subject to disease than plantings in the open, because the aster leafhopper is a wild insect and avoids buildings.

Leafhoppers, because of their activity and rapid multiplication, are difficult to control. It is fortunate, therefore, that only a relatively small number of virus diseases of plants seem to be transmitted by them. It has been known for many years that *Eutettix tenellus* Baker transmits the curly-top disease of sugar beets (1), and that *Nephotettix apicalis* Motsch. spreads the mosaic disease of rice.<sup>2</sup> More recently it has been shown that *Peregrinus maidis* Ashm. transmits the mosaic disease of corn (11), that *Empoa ulmi* L. spreads leaf roll of potato (17), and that *Balclutha mbila* Naude carries the streak disease of sugar cane and corn (29, 30). *Cicadula sexnotata* as the carrier of aster yellows must be added to this list. It is interesting to note that all these leafhoppers except *Peregrinus maidis* belong in the subfamily Jassinae.

A correct understanding of the spread and severity of aster yellows depends largely on a knowledge of the life and habits of *Cicadula sexnotata*. The fortunes of leafhopper colonies vary with weather conditions, with the prevalence of leafhopper diseases, and with the abundance of predacious insect enemies. Under any given set of conditions the incidence and severity of aster yellows are closely connected with the likes and dislikes of this leafhopper. The China aster is very susceptible to aster yellows infection. It is also a favorite host plant of *Cicadula*. These two facts account for the prevalence of yellows in aster plantings. The African marigold, though quite susceptible to infection, is not liked by the leafhopper. This plant may be grown adjacent to badly yellowed aster plots during a whole season without acquiring the disease. It rarely becomes infected when grown in gardens. If, however, a few inoculative leafhoppers are confined in a cage containing only marigold plants, the disease is quickly transmitted. When grown in gardens the plants remain free or almost free of yellows, not because they are resistant to the disease but because *Cicadula* prefers other host plants.

It is thought that the intimate and specific relationship which has been shown to exist between aster yellows and its insect carrier is important evidence in favor of the view that the causative entity is biological rather than chemical. It is difficult to conceive that any agent other than a living organism would require an incubation period in the insect carrier or would be retained by the insect for long periods of time in the absence of susceptible host plants. That the condition of the virus in the insect can influence the length of the incubation period of the disease in the plant suggests the occurrence of developmental changes in the virus during its incubation period in the insect.

<sup>2</sup>Takami, N. Stunt disease of rice and *Nephotettix apicalis*. Jour. Agr. Soc. Japan 241: 22-30. 1901. Mosaic or stunt disease of rice was the first virus disease of plants shown to be transmitted by an insect.

It is interesting to note that the average length of the incubation period of aster yellows in *C. sexnotata* is, at similar temperatures, approximately equal to the length of the incubation period of the malaria organism (*Plasmodium vivax*) in the mosquito *Anopheles*. It is also of the same order as the lengths of the incubation periods shown by *Trypanosoma rhodesiense* in the tsetse fly and by the yellow-fever organism (*Leptospira icteroides*) in the common house mosquito. So far as is known, incubation periods shown by disease-producing organisms in insects are due to a development of these organisms in some tissue or organ of the insect host. It seems probable that the incubation period of aster yellows in *Cicadula sexnotata* is due to a development and multiplication of the causative agent in some tissue of the leafhopper.

#### SUMMARY

1. Aster yellows is a serious disease of the China aster, *Callistephus chinensis* Nees. It is easily distinguished from all other aster diseases. Its characteristics show that it belongs in the virus-disease group.

2. Yellows was transmitted by budding but not by other mechanical means. It is transmitted by the leafhopper *Cicadula sexnotata* Fall. Evidence is brought that it is not transmitted by several other aster insects.

3. By means of this leafhopper the disease has been transmitted to more than 50 different species in 23 different families of plants. In the same way it has been carried from many of these different species back to aster. No evidence has been found that the virus is attenuated by passage through different host plants.

4. Both nymphs and adults are unable to transmit the virus immediately after feeding on yellowed plants. A period of at least 10 days must elapse before they become inoculative. This interval is referred to as the incubation period of the virus in the insect. The incubation period is somewhat shorter in adults than in nymphs. Many individuals retain the virus as long as they live, but some seem to lose it after a short time.

5. Aster yellows is not transmitted through the eggs of the insect carrier or through the seeds of the aster. It is not transmitted from yellowed to healthy plants by contact or directly from a virus-bearing insect to a virus-free insect. Individual insects have been proven to carry the virus for more than 100 days. Small colonies of insects confined on rye plants that are immune to yellows have been shown to retain it for at least 2 months.

6. Aster yellows is identical with white-heart disease of lettuce, with a previously undescribed disease of buckwheat, and with several yellows diseases of cultivated garden plants. It is similar to but apparently distinct from peach yellows, strawberry yellows, curly-top of beets, and false blossom of the cranberry. It is not identical with the stunt disease of Dahlia.

7. The disease overwinters in biennial and perennial host plants, some



of the most common of which belong in the genera *Chrysanthemum*, *Sonchus*, *Asclepias*, *Erigeron*, and *Plantago*.

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### EXPLANATION OF PLATES

#### PLATE XL

*Cicadula sexnotata*. All figures  $\times 14$

- FIG. A. A nymph in the first instar.  
 FIG. B. A nymph in the second instar.  
 FIG. C. A nymph in the fifth instar.  
 FIG. D. A full-grown adult.

#### Aster yellows Disease

FIG. E. The first 36 plants used in determining the incubation period of the virus of aster yellows in insect colony number 3, referred to in table 3. Each plant was exposed for a period of one day. The plant shown in the upper left corner of the figure is the one from which the insects obtained the virus. The next 11 plants remained healthy, showing that the insects were not inoculative during the periods of their exposure. The thirteenth plant shown in the figure was the first to which yellows was transmitted. The insects transmitted yellows to all the 21 plants on which they fed during the 3 weeks following the termination of the incubation period. The picture shows that these 21 plants were badly stunted by the disease. The thirty-fourth and thirty-fifth plants remained healthy, while the thirty-sixth plant became diseased.

#### PLATE XLI

#### Host Range of the Aster yellows Disease

FIG. A. A healthy and a diseased plant of *Chrysanthemum frutescens* L. The diseased plant is stunted and chlorotic.

FIG. B. A healthy and a diseased plant of *Anethum graveolens* L. The diseased plant is dwarfed and chlorotic but has not produced secondary shoots.

FIG. C. A healthy and a diseased plant of *Matricaria alba*. The diseased plant is badly stunted and chlorotic. It has produced many secondary shoots.

FIG. D. A healthy and a diseased plant of *Dimorphotheca aurantiacum* DC. The diseased plant has produced many secondary shoots. It is dwarfed and chlorotic. The petals of the ray flowers are dwarfed and green in color.

FIG. E. A healthy and a diseased plant of *Centaurea margaritae* Hort. The diseased plant is chlorotic and has produced many upright secondary shoots. Some of the diseased leaves are twisted, have irregular margins, and are much longer than normal leaves of the same age.

FIG. F. A healthy and a diseased plant of *Gaillardia aristata* Pursh. The diseased plant is badly dwarfed and chlorotic. It has produced many small secondary shoots.

FIG. G. A healthy and a diseased plant of *Tragopogon porrifolius* L. The diseased plant is chlorotic and somewhat stunted. It has produced many small secondary shoots.

FIG. H. A healthy and a diseased plant of *Taraxacum officinale* Weber. The diseased plant is chlorotic and has produced many secondary shoots. It is not stunted.

FIG. I. A healthy and a diseased plant of *Centaurea imperialis* Hort. The diseased plant is very chlorotic and has produced many upright-growing secondary shoots.

FIG. J. A healthy and a diseased plant of *Reseda odorata* L. The diseased plant is slightly chlorotic and somewhat dwarfed.

FIG. K. A cluster of healthy and a cluster of diseased flowers of *Reseda odorata* L. Secondary flowers have been produced on the stigmas of many of the diseased flowers.

#### PLATE XLII

##### Host Range of the Aster yellows Disease

FIG. A. A healthy and a diseased plant of *Sonchus oleraceus* L. The diseased plant is badly stunted and very chlorotic. Many upright secondary shoots have been produced.

FIG. B. A healthy and a diseased plant of *Gypsophila paniculata* L. The diseased plant is very badly stunted and chlorotic. It has produced many secondary shoots.

FIG. C. A healthy and a diseased plant of *Fagopyrum esculentum* Moench. The diseased plant is slightly chlorotic and somewhat dwarfed. The petals of the flowers on the diseased plant are green in color and usually somewhat reduced in size. A large number of small flowers have been produced.

FIG. D. A healthy and a diseased plant of *Pimpinella anisum*. The diseased plant is stunted and chlorotic. It has produced many fine secondary shoots.

FIG. E. A healthy and a diseased plant of *Ambrosia trifida* L. The diseased plant is dwarfed and has produced many secondary shoots. The young leaves are slightly chlorotic.

FIG. F. A healthy and a diseased plant of *Tagetes erecta* L. The diseased plant is stunted and chlorotic. The flowers are stunted and slightly green in color.

FIG. G. A healthy and a diseased plant of *Schizanthus* sp. The diseased plant is stunted and chlorotic. It has produced innumerable secondary shoots.

FIG. H. A diseased plant of *Calandrina grandiflora* Lindl. which is badly dwarfed and chlorotic. It has produced many secondary shoots.

FIG. I. A healthy plant of *Calandrina grandiflora*.

#### PLATE XLIII

##### Host Range of the Aster yellows Disease

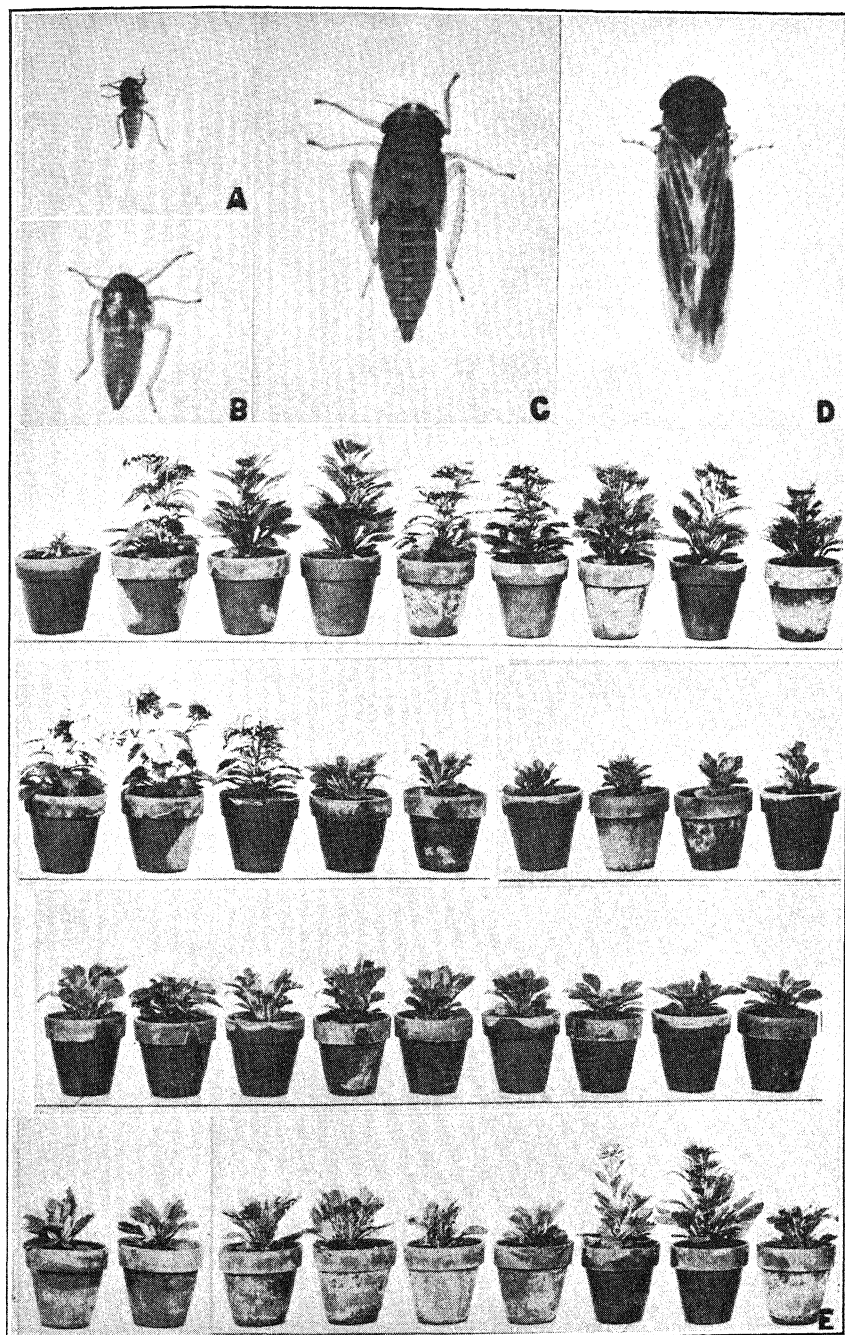
FIG. A. A healthy and a diseased plant of *Ammobium alatum* R. Br. The diseased plant is dwarfed and chlorotic. It has produced many side branches. The leaves are very narrow and have wavy margins.

FIG. B. A healthy and a diseased plant of *Erigeron annuus* (L.) Pers. The diseased plant is chlorotic and much dwarfed. Many secondary shoots have been produced.

FIG. C. Portions of a healthy and of a diseased plant of *Lactuca sativa* L. The diseased plant is shown on the right of the figure. It is chlorotic and shows an upright habit of growth.

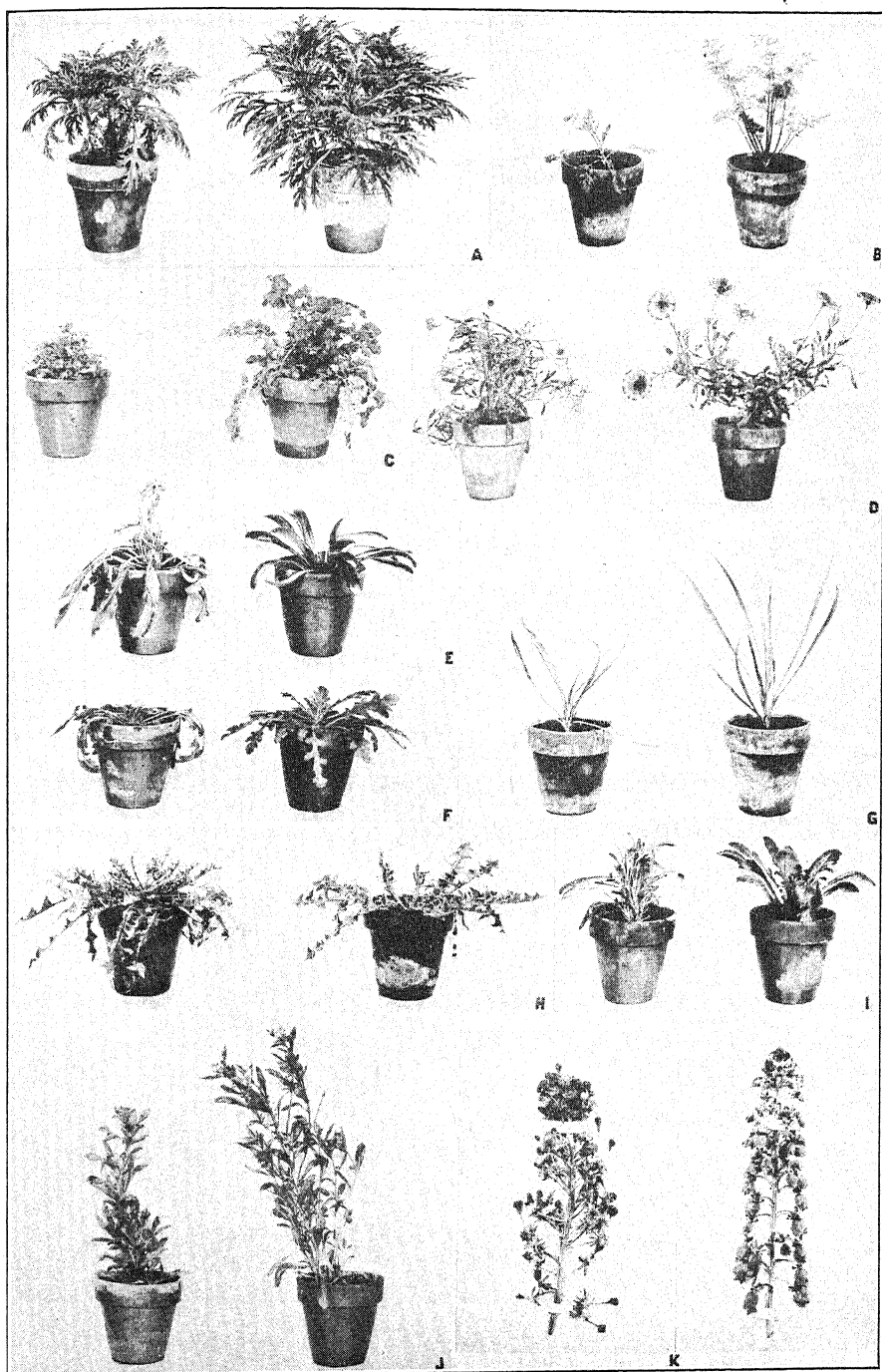
FIG. D. A healthy and a diseased plant of *Asclepias nivea* L. The diseased plant has small, narrow leaves and has failed to bear pods. It is somewhat chlorotic.

FIG. E. A healthy and a diseased plant of *Amaranthus auroro*. The diseased plant is stunted and has failed to produce the red color typical of healthy plants.



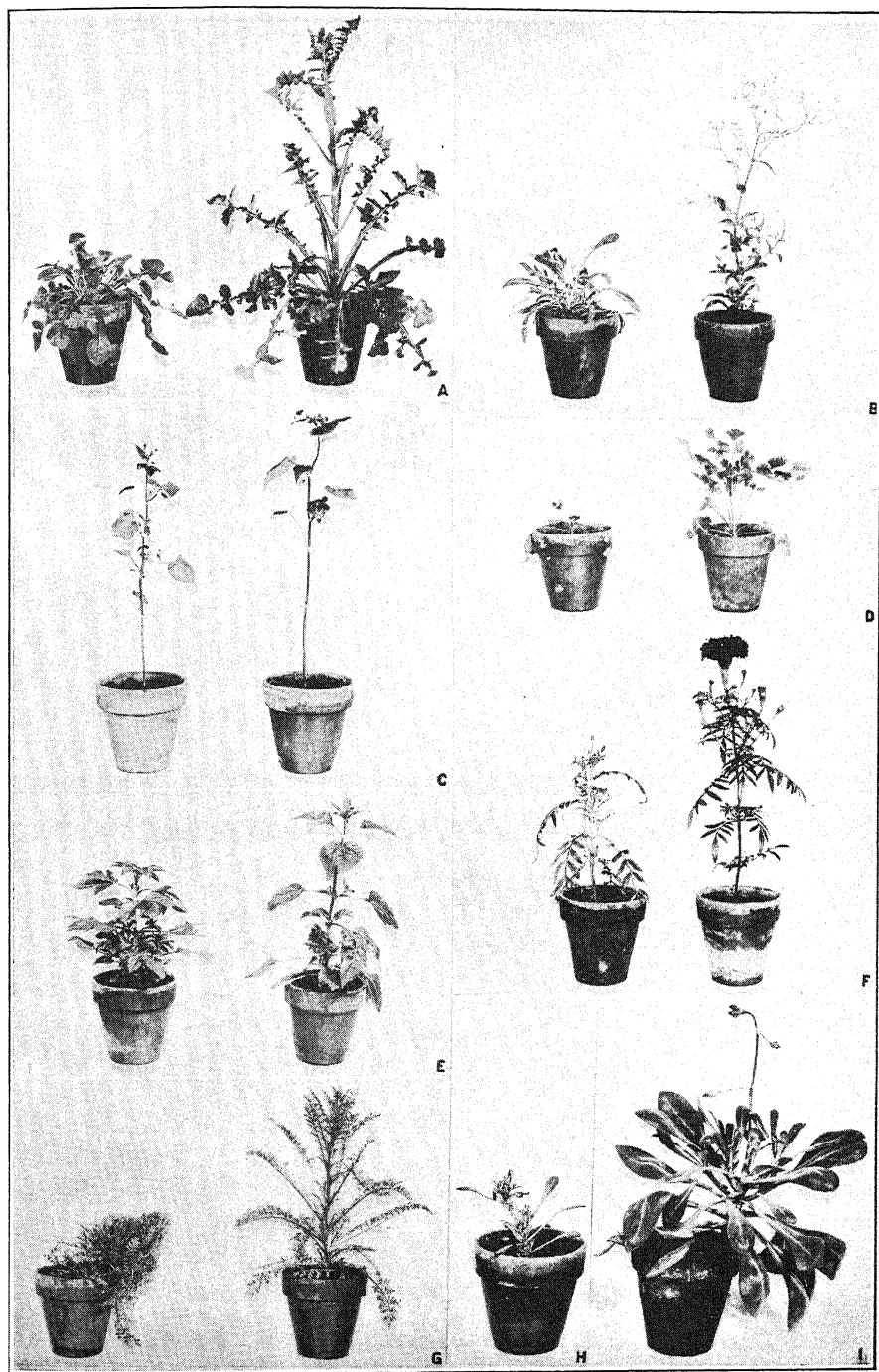
KUNKEL: ASTER YELLOWS





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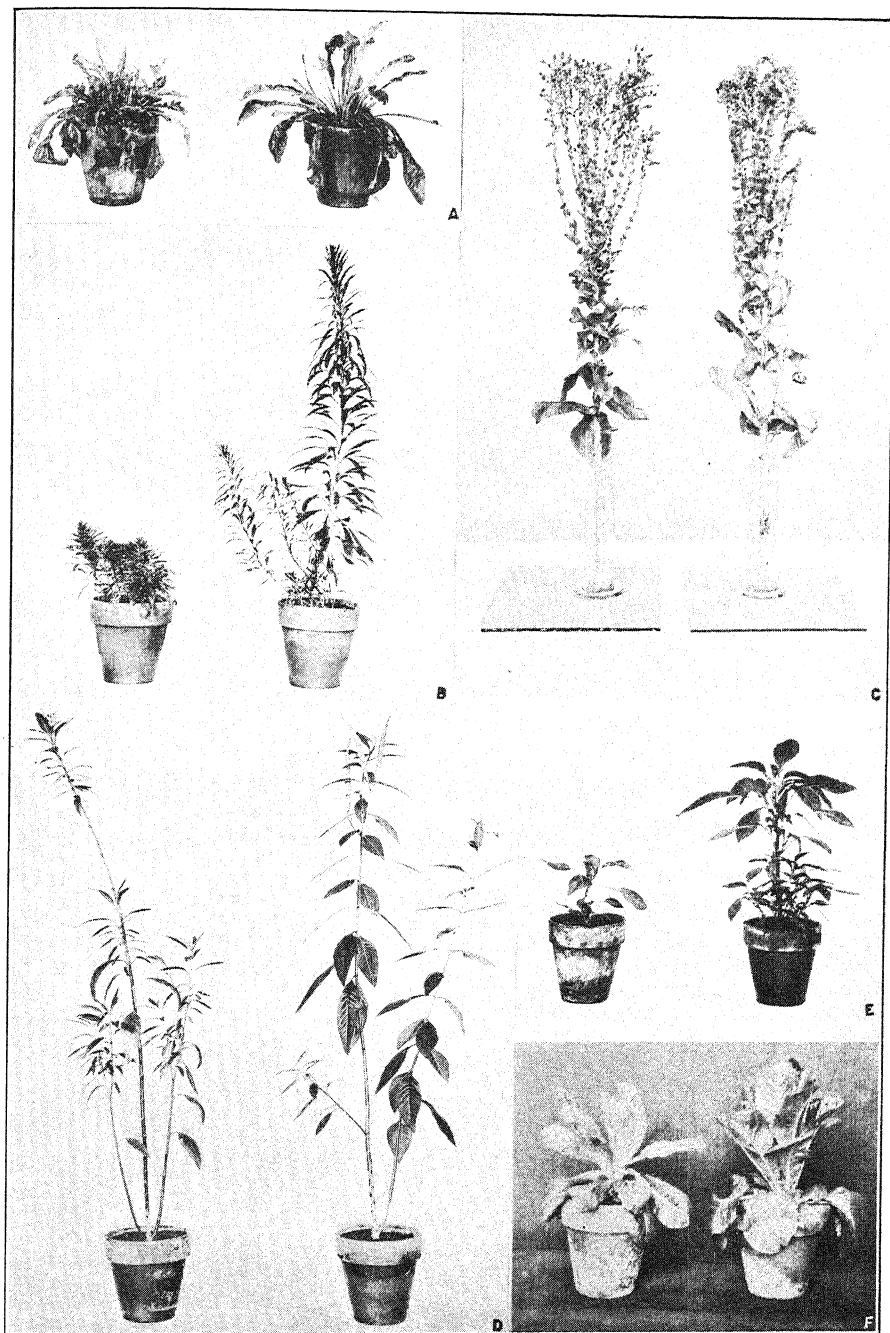




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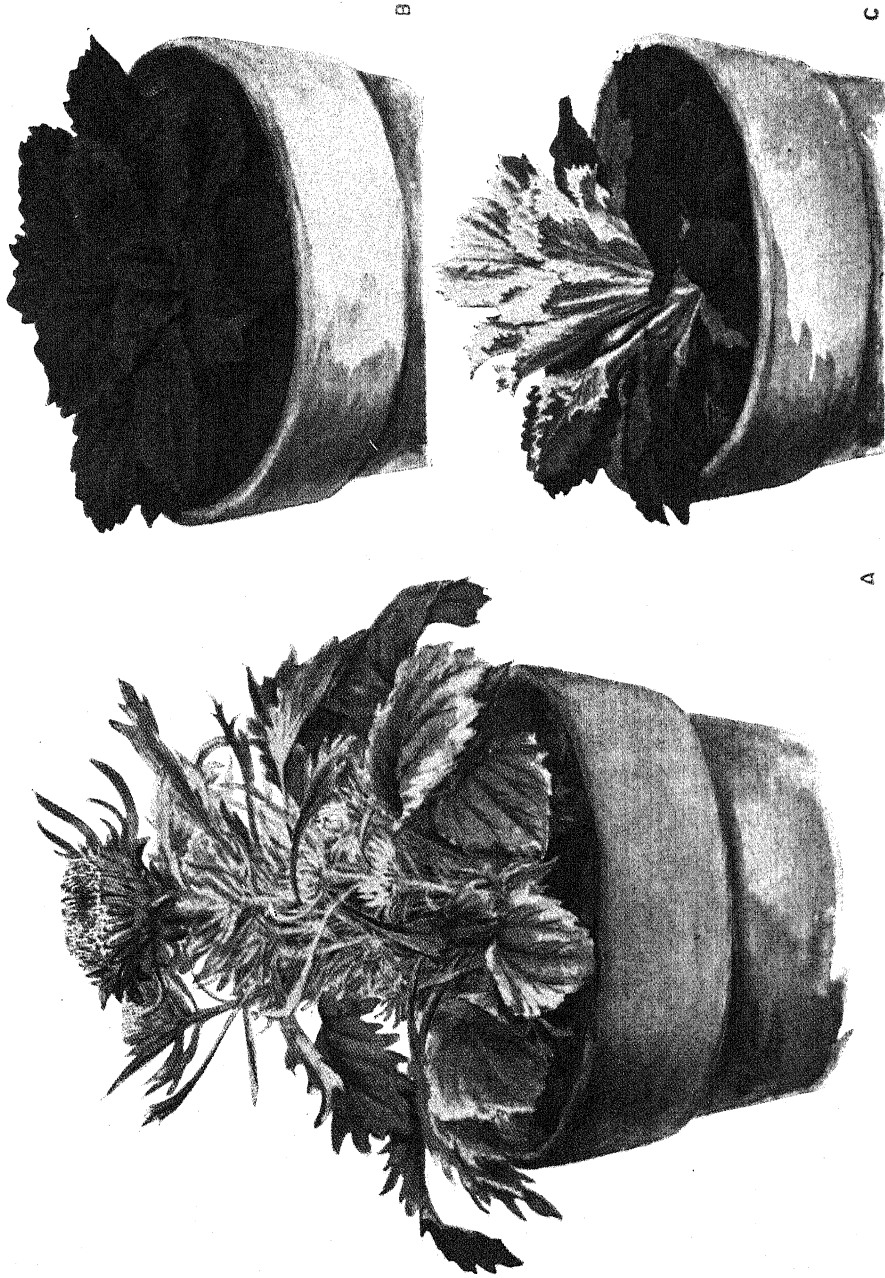






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FIG. F. A healthy and a diseased plant of romaine lettuce. The diseased plant is chlorotic and has failed to head.

PLATE XLIV

Aster yellows Disease

FIG. A. A diseased aster plant in blossom. Yellowed foliage, green-colored flowers, and upright secondary shoots are shown. The plant is badly stunted.

FIG. B. A healthy young aster plant.

FIG. C. A yellowed plant of the same age, showing chlorosis and upright habit of growth of diseased leaves.

# A PHYSIOLOGICAL STUDY OF THE EFFECT OF LIGHT OF VARIOUS RANGES OF WAVE LENGTH ON THE GROWTH OF PLANTS<sup>1</sup>

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## INTRODUCTION

The question as to whether the different parts of the spectrum vary in their effects on plants is an old one. Experiments with plants grown under colored screens were undertaken by Tessier (10) as early as 1783. During the latter part of the nineteenth century many papers appeared dealing with various phases of the subject. Development of chlorophyll and other pigments, rate of elongation of stems, changes in internal structure, plant movement, photosynthesis, and general growth and vigor of plants under different-colored lights were all investigated.

Extensive as the work was during this period, few of the results can be considered with certainty as being directly caused by quality of light because of the failure of the majority of the investigators to take into account variations in light intensity and other factors that affect the growth of plants. With regard to the light, for instance, the only statement given in many cases is that glass of a certain color was used. Very often this color was probably not spectroscopically pure. In fact, many colored glasses, besides cutting down light intensity greatly, transmit the entire visible spectrum. It is obvious, therefore, that a mere statement of the color of the glass used tells us little about the actual quality and intensity of the light falling on the plants.

In recent years improvements in the making of glass to transmit very definite regions of the spectrum have made it possible to conduct more exact experiments in this field. In 1919, Schanz (9) reported the results of his experiments with plants grown under light consisting of definite regions of the spectrum of daylight. He arranged his plants in eight beds which were covered with various kinds of glass. In the first five beds the range of wave lengths of light transmitted was gradually decreased from the violet end of the spectrum toward the red, thus enabling him to study the effect on plants of light from which greater and greater regions of the spectrum were eliminated in the blue-violet end. In the last three beds he used combinations of colored glasses which gave predominating colors of yellow, green, and blue-violet respectively.

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research. Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

In general, he found that plants became taller the more the short rays of sunlight were cut off from them. Maximum height was obtained under red light and minimum under blue-violet. This was true for cucumbers, Fuchsia, Chrysanthemums, Lobelia, Begonia, and Oxalis; but potatoes and red beets were weakest in yellow light, a little stronger in green light, and still larger and healthier in blue-violet light.

Chlorophyll-development in beans, soybeans, and potatoes was more rapid the more the short rays were cut off, being most rapid under red light. In lettuce, chlorophyll could not be formed in normal quantity in yellow or in green light but did develop fully under blue-violet rays. Epidermal anthocyanin of the leaves of certain plants failed to develop under any glass that eliminated ultra-violet rays. Flowers also became paler in color the more the short rays were removed.

With such plants as Fuchsia, beans, and tomatoes, the time of flowering was hastened gradually and the number of flowers and fruits increased as the short rays were cut off, but under more limited regions of the spectrum, namely, under red, yellow, green, or blue-violet light respectively, the number of flowers was greatly reduced and the time of flowering was postponed. The germination of nettle seeds was favored by the elimination of the short wave lengths.

As a general result of his work, Schanz concluded that light of short wave lengths, particularly ultra-violet rays, was detrimental to the growth of plants. He therefore recommended the use of Euphos glass, which eliminates these rays, for greenhouses.

Schanz's work was superior to that of many of his predecessors in that he knew the exact ranges of wave lengths of light used. He did not, however, make any measurements of light intensity, nor did he give us accurate information concerning temperature and other factors that no doubt varied under the different types of glass. We were thus again left in doubt as to whether his results were really caused by the quality of light.

Klebs (6), in a careful study of the developmental physiology of fern prothallia, has shown that very striking formative changes can be induced in prothallia by different parts of the visible spectrum. He also showed, however, that intensity and duration of light as well as other environmental factors may bring about similar effects, which fact demonstrates the importance of measuring or recording all these factors in any study of the effect of light on plants. None of the higher plants was used in his investigation.

The general uncertainty of the results obtained by previous investigators and the importance of the question of light relations of plants led the Boyce Thompson Institute for Plant Research early to install such equipment as would enable workers to attack this problem under better-controlled conditions and on a scale that has hitherto not been attempted. The present paper is concerned with the results of the first investigation at this

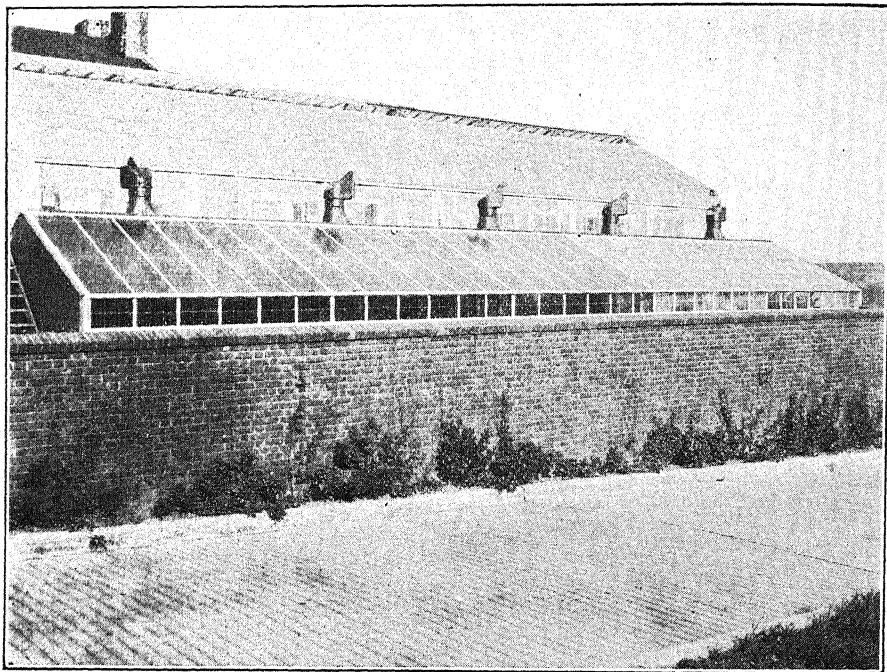


institution on the effect of various ranges of wave lengths in the sun's spectrum on the growth of plants.

## MATERIALS AND METHODS

### Greenhouse Construction

The investigation was carried out in five small, adjacent greenhouses constructed especially for this type of work. Each house was 11 feet long and 9 feet wide. The houses had a continuous roof of glass sloping toward the south. This roof was  $3\frac{1}{2}$  feet above the benches in the front of the houses and  $5\frac{1}{2}$  feet above them behind. A photograph of these houses is given in text figure 1. For convenience in referring to them the houses are



TEXT FIG. 1. Greenhouses in which experiments were conducted. The copper ventilators shown in the picture are above the middle of each house. A recording pyrheliometer is attached to the ventilator of house 1, on the extreme right.

numbered 1 to 5, beginning at the right of the picture. A complete set of plants was also grown for comparison outside, adjacent to the five houses. This outside plot is referred to as house 6. Detailed observations of the plants in this outside plot are not included in this paper because of the great differences in general environmental conditions between it and the first five houses.

The houses were separated from each other on the inside by means of

metal partitions extending several feet below the benches, but were all intercommunicating below so as to allow free circulation of air. A large electric fan was installed underneath the benches of the east-end house (house 1), which communicated with all the houses by means of a continuous tin ventilator. Each house had two inlet pipes provided with dampers by the regulation of which the circulation of air and the temperature could be kept the same in all houses. In addition, each house was provided with a revolving copper ventilator on the north roof, as shown in text figure 1. The entire interior of each house was painted white.

### Temperature

The temperature of each house was automatically recorded by means of Cambridge and Paul electric thermographs. At any given time the temperature did not vary more than 2 to 4° C. in the different houses. The average temperatures during the growth period of the experiment for all houses are given in table 1.

TABLE 1. *Average Temperatures in Degrees C.*

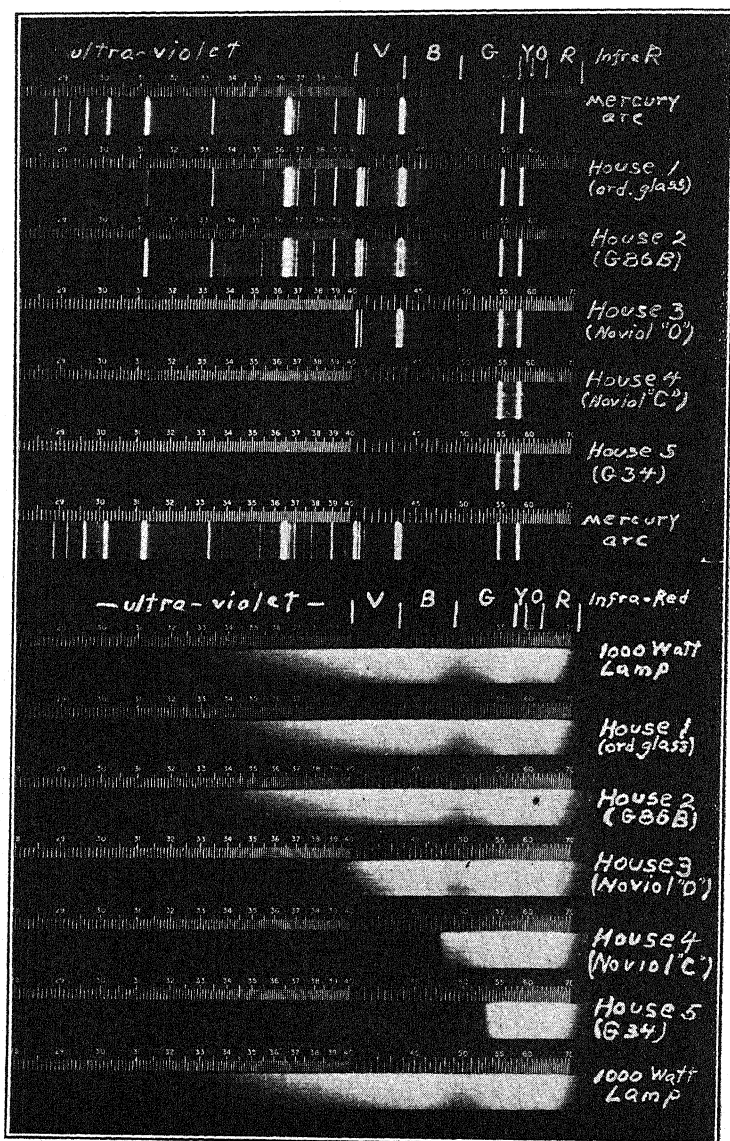
Week	Day		Night	
	Average Maximum	Average	Average Minimum	Average
Sept. 3-9.....	31.6	25.6	17.2	18.3
Sept. 10-16.....	31.1	25.0	16.7	17.8
Sept. 17-23.....	27.8	22.8	15.6	17.8
Sept. 24-30.....	28.3	23.3	13.9	16.1
Oct. 1-7.....	33.3	27.2	16.1	17.8
Oct. 8-14.....	32.2	27.2	12.2	14.4
Oct. 15-21.....	31.6	26.7	13.3	15.6
Oct. 22-28.....	31.6	25.6	17.2	18.9
Oct. 29-Nov. 4.....	28.9	25.0	18.3	19.4
Nov. 5-11.....	28.3	25.0	18.3	19.4
Nov. 12-18.....	28.9	23.3	15.6	16.7
Nov. 19-25.....	26.7	22.8	16.7	17.8
Nov. 26-Dec. 2.....	27.8	22.8	15.0	16.1
Dec. 3-9.....	25.0	21.1	16.7	17.8

The figures in table 1 were obtained by averaging the daily and hourly temperatures during the growth period. As will be seen from the table, no attempt was made to keep the temperature constant. All plants in all houses, however, were subjected to the same fluctuations of temperature, thereby eliminating temperature difference as a factor in the results.

### Light Conditions

Since it is practically impossible to maintain on a large scale an artificial light source that is comparable to daylight in both intensity and quality, the source of light used was ordinary daylight from which different parts of the spectrum were screened out by the use of different kinds of glass.

House 1 was covered with ordinary greenhouse glass and was used for comparison. The glass over the other houses was made by the Corning Glass Company for this particular work and transmitted only definite regions of the spectrum.



TEXT FIG. 2. Spectra of glasses used in greenhouses; upper series taken with a mercury-vapor arc lamp in quartz; lower series taken with a 1000-watt Edison Mazda lamp. The line at 296 millimicrons transmitted by the glass of house 2 does not appear distinctly on the print.

A quartz spectrograph was used to determine the actual range of wave lengths each glass transmitted. A print showing these ranges is given in text figure 2. Both a mercury-vapor lamp in quartz and a 200-watt lamp were used as light sources for making the spectrograms; the former because it is rich in ultra-violet rays and the latter because, having a continuous spectrum, it enabled one to determine the exact limits of those glasses the cut-off of which was in the visible region. In table 2 are given the exact ranges of wave lengths in the visible and ultra-violet transmitted by the various glasses used, together with the limits usually assigned to the different colors of the visible spectrum. It is assumed that the infra-red region begins at 720  $\mu\mu$ .

TABLE 2. *Spectral Limits of Glasses and of Regions in the Visible and Ultra-violet*

Spectral Regions and Glasses	Spectral Range in Millimicrons ( $\mu\mu$ )
Sunlight.....	290-720
Ultra-violet of sunlight.....	290-400
Violet.....	400-435
Blue.....	435-490
Green.....	490-574
Yellow.....	574-595
Orange.....	595-626
Red.....	626-720
Ordinary greenhouse glass (house 1).....	312-720
G 86 B (house 2).....	296-720
Noviol "O" (house 3).....	389-720
Noviol "C" (house 4).....	472-720
G 34 (house 5).....	529-720

In order to determine the relative intensity transmission of the glasses used in the different houses, the light intensities were measured inside the houses by means of a Macbeth illuminometer. House 2, which transmitted practically the entire spectrum of daylight, had to be shaded on the inside by means of an 8  $\times$  8 mesh tobacco-shading cloth to reduce its intensity to a figure comparable to that of the houses having a limited spectrum. The approximate transmission of the glasses, as given in table 3, was determined

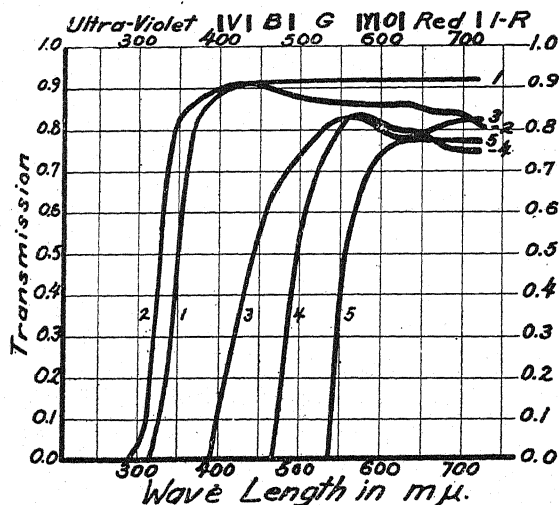
TABLE 3. *Relative Transmission of Total Intensity of Light by Glasses in Different Houses*

House Number	Approximate Transmission %	Actual Intensity 10:00-11:00 A.M. Oct. 2, 1924 (foot-candles)
Outside.....	100.0	5833
1.....	80.0	4615
2.....	46.6	2795
3.....	66.1	3756
4.....	56.7	3372
5.....	37.0	2199

by taking the average of all the measurements of actual intensities in the different houses at different times throughout the time of the experiment and

computing the transmission on the basis of the average outside intensities at the same times. In the same table is given a sample record of the actual intensities as measured with a Macbeth illuminometer in the different houses between 10:00 A.M. and 11:00 A.M. on October 2, which was a very clear day.

It is important to note here that, while the intensities were not exactly the same in the different houses, the intensity in house 2, which transmitted the entire spectrum of daylight, was at all times lower than that in house 4 and only slightly greater than that in house 5. The latter two houses, as will be seen in text figure 2 and table 2, had the narrowest ranges of wave



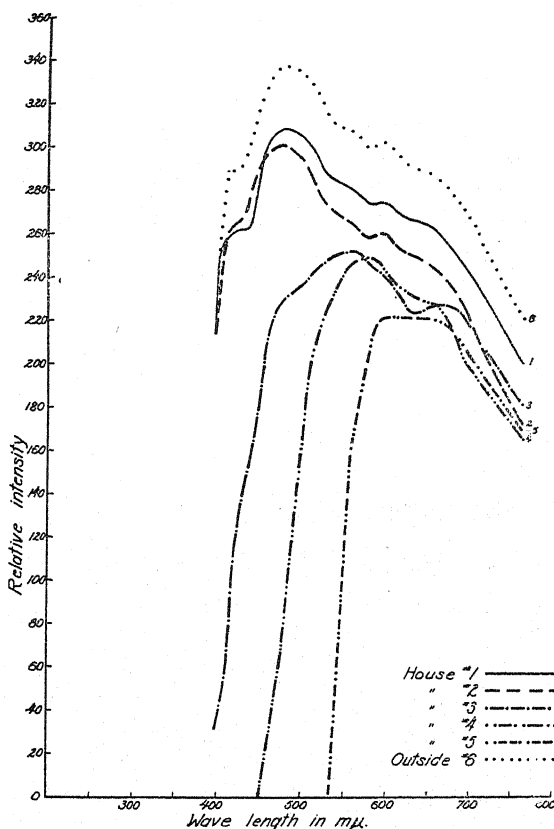
TEXT FIG. 3. Transmission curves of glasses in the visible and ultra-violet. Figures on curves represent house numbers; 1 is ordinary greenhouse glass (house 1); 2 is Corning glass "G86B" (house 2); 3 is Corning's Noviol "O" (house 3); 4 is Corning's Noviol "C" (house 4); 5 is Corning glass "G 34" (house 5).

lengths in the spectrum. It is apparent, therefore, that intensity differences could not be a controlling factor in the final results obtained in these houses.

Since the intensity of daylight varies from hour to hour and from day to day, no attempt was made to keep a constant record of it by measurements with a Macbeth illuminometer. Since, however, the exact transmission of the glasses is known, and since the U. S. Weather Bureau keeps a constant record of intensity at its New York City station, which was only a few miles away, such records are available. These records are given in gram calories per square centimeter per minute. Kimball (4) has found that if these figures (gram cal. per cm.<sup>2</sup> per min.) are multiplied by the factor 6700, the resulting figure will give the illumination intensity in foot-candles. From the latter figure the intensity in any of the houses can be computed by simply multiplying by the transmission of the glass in that

house. It is therefore possible to determine the actual illumination intensity for any given time in any house throughout the growth period.

Text figure 3 gives the transmission curves of the various glasses in all regions of the visible and ultra-violet. These curves were constructed from curves obtained for the various types of glass and corrected for the thickness of the actual glasses used. Curves showing the relative intensity of different wave lengths of light as received on a horizontal surface in the open from sun and a cloudless sky at different hours at latitude  $41^{\circ}$  N. on September 21



TEXT FIG. 4. Relative energy-distribution in the visible spectrum of the light transmitted by the glass in the different houses, as determined for noon, September 21, on a clear day. The curve for house 2 does not take into account the reduction in intensity equally at all wave lengths, caused by the tobacco-shading cloth used in this house, which causes the entire curve to be shifted to a lower position but does not change its shape.

have been made by Kimball (5). Similar curves are available for other seasons of the year, but these particular curves would hold approximately for the period during which the experiment was conducted. By applying to these curves the percentage transmission of the different glasses at

different wave lengths, as given in text figure 3, curves are obtained which give the distribution of the energy in the visible spectrum of the light transmitted by each of the types of glass. A series of such curves, determined for each kind of glass used in the different houses, for noon, September 21, is given in text figure 4.

### Plants and Culture Methods

The following plants were grown in each house: Tobacco (*Nicotiana tabacum*) variety Havana, 5 pots; carrots (*Daucus carota*) variety Danvers Half Long, 4 pots; petunia (*Petunia hybrida*?) variety Balcony Crimson, 4 pots; sunflower (*Helianthus cucumerifolius*), a small variety, 5 pots; soybeans (*Soja max*) variety Peking, 6 pots; four o'clocks (*Mirabilis Jalapa*), 2 pots; coleus (*Coleus Blumei*), variegated, 2 pots; buckwheat (*Fagopyrum vulgare*) variety Japanese, 2 pots; tomato (*Lycopersicum esculentum*) variety Bonny Best, 2 pots; Sudan grass (*Holcus sorghum sudanensis*), 4 pots.

All plants were grown in 2-gallon jars, perforated at the bottom. The soil used consisted of a top soil taken from a forest, to which was added horse manure at the rate of one part of manure to three parts of soil, and a liberal amount of potassium sulfate, acid calcium phosphate, sheep manure, and lime, the whole being thoroughly mixed. All pots were filled from the same batch of soil.

The plants were so distributed within the houses as to avoid differences resulting from shading. Whenever comparisons were made between plants in the different houses, or when photographs were taken, the plants were always taken from the same relative position in each house.

The experiment was begun on August 26, 1924. The Sudan grass, soybeans, four o'clocks, and buckwheat were planted on this day. The tobacco plants were about one inch high when placed in the houses on August 26. They had been sown August 1, 1924, and were above ground August 9. The carrots and sunflowers were sown August 22 and were not yet up when the pots were placed in the houses. The petunia plants, sown July 21, were 2 to 3 inches high, the coleus plants (July cuttings) were 2 to 4 inches high, and the tomato plants, sown July 26, 5 to 7 inches high when placed in the houses. Tobacco, petunias, four o'clocks, coleus, and tomatoes were grown one plant per pot. Sunflowers were thinned to 2 or 3 plants per pot, soybeans and buckwheat to 6 plants per pot, and carrots and Sudan grass to about 12 to 15 plants per pot.

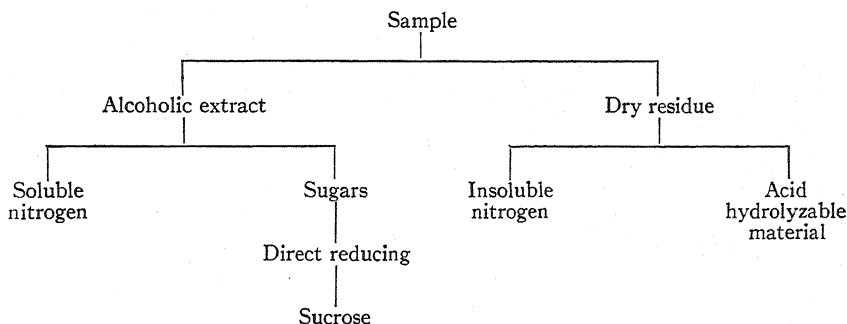
Besides general observations on vegetation, flowering, and fruiting, weekly measurements of height of all plants and chemical analyses were made, and anatomical and other differences were followed.

### Methods of Chemical Analysis

The plants to be analyzed were placed in a dark room over night and then removed to a refrigeration room and allowed to remain until frozen.

Usually they were left in the refrigeration room one to two days and never less than 3 hours. The frozen plants were then ground and mixed thoroughly. Two portions were removed to paired watch glasses to be used for moisture determinations. Two more portions, varying from 40 to 60 grams each, were placed in tared 250-cc. Erlenmeyer flasks into which one to two tenths of a gram of  $\text{CaCO}_3$  was added. These were boiled in 95-percent alcohol for 10 minutes, then made up to 200–225 cc., stoppered, sealed with paraffin, and set away in the dark until they were to be used for analysis.

The samples were extracted three times with 50-percent alcohol. The dried residue and the extract were used for determinations of carbohydrates and nitrogen according to the following outline:



Each analysis reported is the average of two separate samples carried through the complete series of analyses.

Insoluble nitrogen was run on a two-fifth aliquot of the residue, using the Official Method modified to include nitrates. Soluble nitrogen was run on a two-fifth aliquot of the alcoholic extract, using also the Official Method modified to include nitrates. Total nitrogen as expressed in the tables was obtained by adding together the results of the two preceding determinations.

Direct reducing sugar and sucrose were determined on two fifths of the alcoholic extract after removing the alcohol by evaporation, treating with saturated lead acetate, and deleading with potassium oxalate. The official Munson and Walker method was used for determining the reducing sugar, and the results were calculated as dextrose. Sucrose was determined by first calculating the results, after treatment with concentrated  $\text{HCl}$ , as invert sugar, then subtracting from this the direct reducing value and multiplying by 0.95.

The acid hydrolyzable material was determined on two-fifths portions of the dried residue, reducing sugar being determined on the material after hydrolysis with  $\text{HCl}$ , the results were calculated as dextrose, and then multiplied by the factor 0.9.

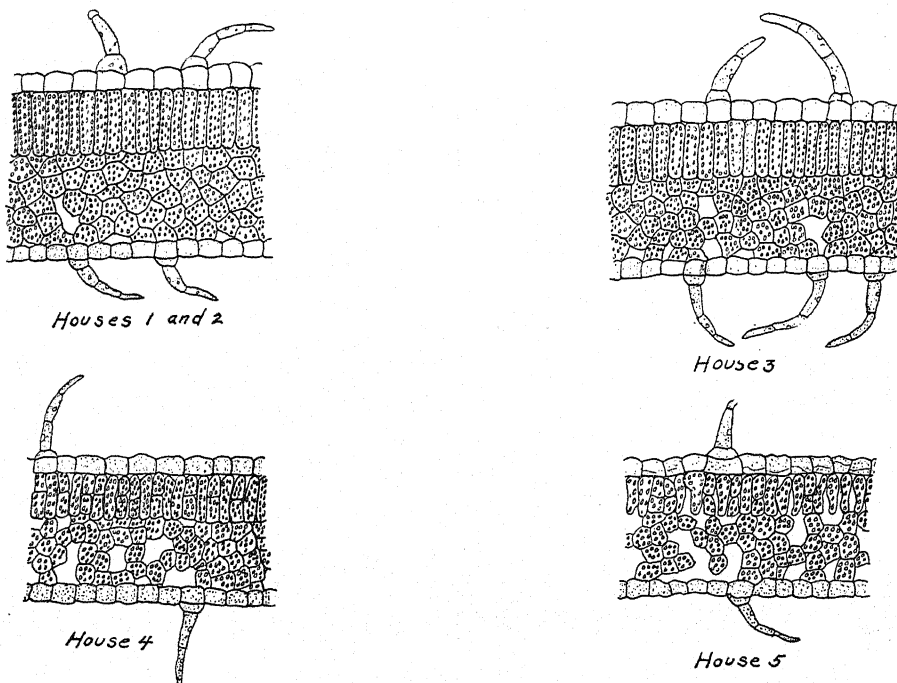
In addition to these chemical determinations, microchemical tests for carbohydrates and nitrates were made from time to time during the progress of the work.



## RESULTS

## General Growth and Development

The most striking results in growth and development were obtained in those houses (4 and 5) in which the entire blue-violet end of the spectrum was eliminated. The plants in these houses were decidedly etiolated, although they had a good green color which in some cases, like the tomato, was even darker than that of the plants in the other houses. The stems were generally long and slender, with greatly elongated internodes and few branches. Internally these stems had a rather loose structure with little differentiation of tissues and weak development of secondary and strengthening tissues. The cells were thin-walled. Practically all the plants had to be supported after two or three weeks' growth. Soybeans completely changed their habits of growth in these houses and became twiners (Plate XLV, fig. 3). The leaves did not vary greatly in size from those of plants in the other houses, but they showed a general tendency toward crinkling or rolling which was rather pronounced in sunflower, petunia, tomato, tobacco, and four o'clocks. Leaves of tobacco were somewhat longer and narrower in these two houses. Internally, the leaves, like the stems, showed a loose structure with little differentiation of tissues. There were large air spaces.

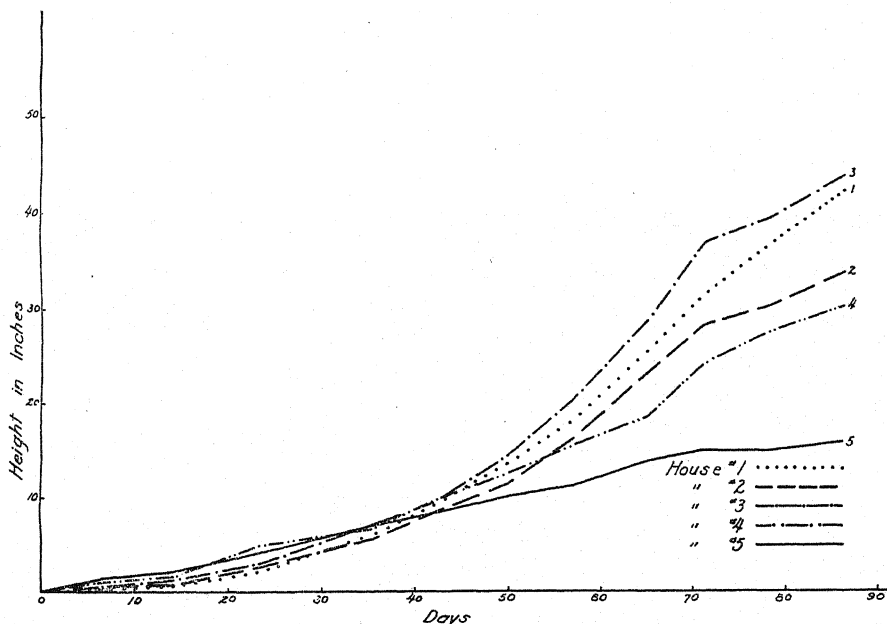


TEXT FIG. 5. Internal structure of tomato leaves in the different houses. All drawn to same scale.

The cells were thin-walled and loosely arranged even in the palisade region. Text figure 5 shows some of these features as they appeared in the tomato. The cells of the mesophyll were generally full of chloroplasts and were not greatly different in this respect from those of plants in the other houses.

The leaves of variegated coleus, which in the first three houses were of a dark red color, were much paler in houses 4 and 5. Normally there is a very narrow green margin on these leaves. This margin became much wider in houses 4 and 5, and particularly in house 5. The color of the flowers of tobacco, four o'clocks, and petunia was also somewhat paler in houses 4 and 5.

With the exception of the soybeans, all the plants in houses 4 and 5 were delayed in reaching maturity as compared with the plants in the other houses. The pods of soybeans in houses 4 and 5 began to dry out and ripen in 70 days, and then no new pods were developed. The leaves also began to turn yellow at this time. In the other houses, new pods kept forming and the plants remained vigorous and green to the time they were sampled for analysis (91 days).

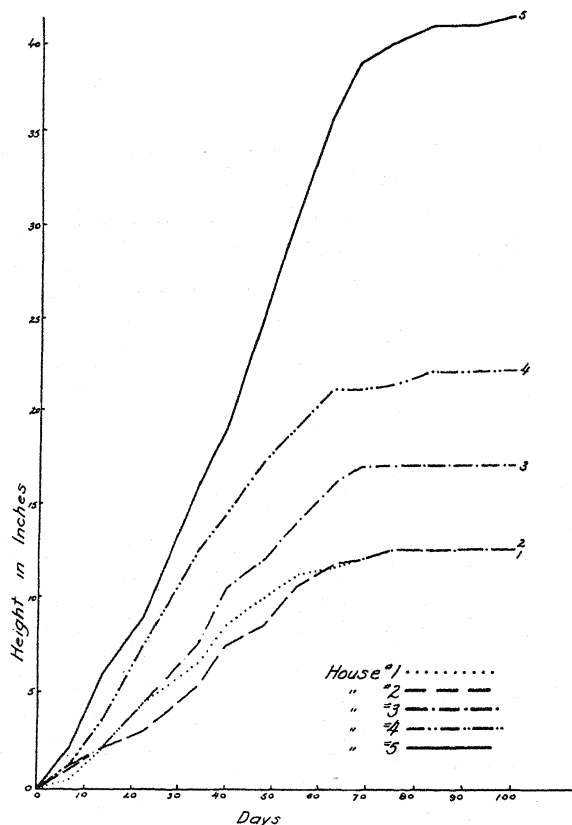


TEXT FIG. 6. Growth in height of sunflowers in the different houses.

Removal of only the ultra-violet rays (house 3) had little effect on the general growth and appearance of the plants as compared with those receiving these rays (houses 1 and 2). The plants were slightly taller and in some cases bloomed earlier, but otherwise no great differences were observed. All plants had a vigorous and healthy appearance in the first three houses.

### Stem Elongation

No differences were observed in the rate of germination of seeds in the different houses. All plants that were sown in the houses came up on the same day in all the houses. As soon as they appeared above ground, however, the seedlings began to show differences in the rate of growth. For



TEXT FIG. 7. Growth in height of four o'clocks in the different houses.

the first 2 or 3 weeks the rate of stem-elongation was uniformly greatest in all species in houses 4 and 5, which had the narrowest range of wave lengths in the spectrum (table 4 and text figures 6-8). After that time the rate in these two houses fell below that of the plants in the other houses in the cases of Sudan grass, petunia, sunflower, and buckwheat. In all these species the shortest stems at the end of the experiment were found in house 5 and the next shortest in house 4.

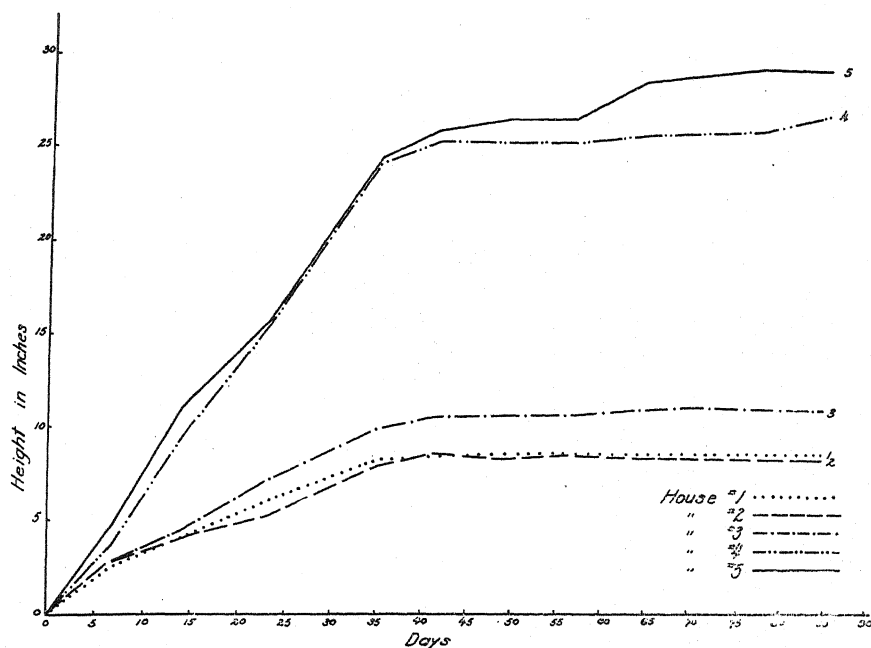
There was little difference in the rate of growth of tobacco in the different houses during the first 40 days. For a short period after this the plants in houses 4 and 5 fell behind those of the first two houses. It was at this time

TABLE 4. *Average Height of Plants in Inches, Measured from Base of Stem to Highest Point*

Plant	House No.	Hgt. at Start	Days in Houses										No. Pots			
			7	14	23	35	41	49	56	64	70	77	85	94	102	
Tobacco.....	1	1.0	1.5	2.5	6.4	9.4	12.1	19.2	31.7	37.2	36.5	36.5	36.5	36.5	36.5	5
“.....	2	1.0	1.5	2.6		9.1	12.4	20.1	32.3	39.2	38.5	38.5	38.5	38.5	38.5	5
“.....	3	1.0	1.5	2.6	6.0	8.5	11.4	16.2	28.5	41.0	44.3	44.5	45.5	45.5	45.5	5
“.....	4	1.0	1.5	2.6		8.5	9.7	14.3	21.8	35.5	40.3	41.5	41.5	42.0	42.0	5
“.....	5	1.2	1.8	2.8	6.6	10.7	12.5	17.6	25.3	42.7	48.3	49.5	49.5	49.5	49.5	5
Sudan grass.....	1	0.0	3.0	5.2	13.5	19.9	22.4	28.5	30.7	40.2	49.1	54.1	59.0			4
“.....	2	0.0	2.7	5.4	10.4	15.9	19.2	25.2	30.9	43.2	51.5	56.7	61.1			4
“.....	3	0.0	2.8	5.9	11.5	19.0	22.9	28.9	36.6	46.5	53.2	60.4	61.4			4
“.....	4	0.0	3.2	6.0	9.0	18.0	20.5	26.4	39.7	40.7	43.6	44.7	48.0			4
“.....	5	0.0	3.5	6.2	9.7	17.6	19.5	24.0	29.1	36.9	37.2	38.5	42.5			4
Petunia.....	1	2.5	2.9	3.2	4.1	5.0	7.1	8.5	14.4	18.9	22.7	26.5	29.0	31.7	34.2	4
“.....	2	2.7	3.1	3.6	5.6	7.0	9.4	12.4	15.6	20.1	22.9	27.5	28.6	31.0	31.6	4
“.....	3	3.0	3.4	4.2	6.1	9.4	11.6	15.0	17.2	23.0	28.7	33.1	33.4	35.6	35.6	4
“.....	4	2.7	2.8	3.7	4.7	6.2	7.7	10.1	12.7	18.4	19.7	21.3	21.7	23.7	24.4	4
“.....	5	2.8	4.0	5.9	8.4	9.4	9.5	12.0	16.6	17.7	23.5	23.6	23.6	25.7	27.6	4
Sunflower.....	1	0.0	0.4	0.6	2.0	5.7	8.8	13.4	17.9	25.0	30.8	36.0	41.6			5
“.....	2	0.0	0.5	0.7	2.5	5.3	8.0	11.3	16.0	22.6	27.6	29.6	33.2			5
“.....	3	0.0	0.5	1.1	2.9	6.5	9.2	14.0	20.0	28.3	36.1	38.7	43.2			5
“.....	4	0.0	0.8	1.5	4.9	6.5	9.4	12.3	15.2	18.2	23.6	27.0	29.7			5
“.....	5	0.0	1.3	2.0	4.0	6.9	8.0	9.8	10.1	13.7	14.7	14.8	15.9			5
Tomato.....	1	6.9	8.0	10.1	14.5	22.4	26.2	32.0	37.2	43.0	43.8	43.9	44.0			2
“.....	2	6.9	7.7	10.2	14.0	21.7	24.5	29.5	35.7	44.0	45.7	46.0	46.7			2
“.....	3	6.2	7.7	10.3	16.5	23.9	26.7	30.8	36.5	46.5	49.7	50.5	51.0			2
“.....	4	6.2	7.7	10.0	17.0	28.2	35.2	41.7	46.0	54.5	56.8	57.0	57.5			2
“.....	5	6.2	9.5	15.1	23.0	37.5	40.5	49.5	54.0	54.5	59.0	59.2	59.3			2



that the plants of the first two houses were rapidly sending up a flowering shoot, which caused the height of the plants to increase rapidly for a period of about 10 days. The plants in houses 3, 4, and 5 did not flower until 5, 6,



TEXT FIG. 8. Growth in height of soybeans in the different houses.

and 14 days, respectively, later, and therefore continued to grow in length after the plants in the first two houses ceased growth altogether. At maturity the tobacco plants in house 5 were the tallest, followed by those in house 3 and house 4 respectively.

Tomatoes, soybeans, four o'clocks, and coleus (Pl. XLV) produced the tallest plants of these species in the series in houses 4 and 5. The results were particularly striking with soybeans and four o'clocks (text figures 7, 8; Pl. XLV, figs. 2, 3). The soybeans at maturity were three times as tall in houses 4 and 5 as those in houses 1 and 2, and the four o'clocks were twice as tall in house 4 and over three times as tall in house 5. This increased height was in each case due to increases in length of all internodes and not to an increase in the number of internodes, as shown in table 5.

Removal of only the ultra-violet rays of daylight (house 3) also resulted in a somewhat increased rate of elongation in all species studied. With Sudan grass, petunias, and sunflowers the tallest plants of the series were produced under these conditions, and the plants of tobacco, tomato, soybeans, four o'clocks, and coleus were all taller than those receiving the full spectrum of daylight.

TABLE 5. *Average Lengths of Internodes of Soybeans (92 Days)*  
(Average of 32 Plants in Each House)

House No.	Internodes (Length in Inches)										Total Length of Stem
	Hypocotyl	1st	2d	3d	4th	5th	6th	7th	8th	9th	
1.....	1.47	1.39	0.75	1.01	1.26	0.98	0.57	0.28	0.10	0.03	7.84
2.....	1.69	1.38	0.77	1.06	1.39	0.86	0.42	0.17	0.01		7.75
3.....	1.68	1.97	1.13	1.45	1.58	1.10	0.57	0.18	0.05	0.01	9.73
4.....	2.62	5.20	4.00	5.25	6.37	3.49	0.87	0.12	0.01		27.93
5.....	3.35	6.39	4.83	6.34	5.73	2.12	0.37	0.03			29.16

### Stem Thickness

Removal of the blue-violet end of the spectrum (houses 4 and 5) uniformly resulted in decreased stem thickness. This was true both for plants that became tallest under these conditions and for those which remained the shortest of the series. Table 6 shows the thickness of soybean stems in the different houses.

TABLE 6. *Diameters of Stems of Soybeans (92 Days)*  
(Average of 10 Plants in Each House)

Inches					
House No.	Internodes				Average of all Internodes
	Hypocotyl	2d	4th	6th	
1.....	0.133	0.244	0.176	0.117	0.167
2.....	0.144	0.263	0.227	0.137	0.193
3.....	0.133	0.202	0.168	0.118	0.155
4.....	0.112	0.104	0.058	0.064	0.085
5.....	0.074	0.061	0.043	0.036	0.054

A similar condition was found in other plants in the different houses. Thus, sunflowers of house 1 had an average stem thickness of 0.41 inch; those of house 2, 0.42 inch; those of house 3, 0.39 inch; those of house 4, 0.25 inch; and those of house 5, 0.12 inch. The stoutest stems were uniformly found in houses 1 and 2, although in general the difference between the thickness of the stems in these houses and those in house 3 was not great.

The general weakness of the stems in houses 4 and 5 necessitated the supporting of these plants long before this was necessary in the other houses. The soybeans, after being in the houses 35 days, began to twine around the supports in houses 4 and 5. No twining occurred, however, in the other houses, nor did twining occur in any other species in houses 4 and 5 in spite of the fact that many of the stems were excessively long and slender and were supported in the same manner as the soybeans.

### Flowering, Fruiting, and Storage

Removal of the blue-violet end of the spectrum had a marked effect in some species on the time of flowering of the plants, as shown in table 7. The plants of houses 4 and 5 uniformly flowered later than those of the other houses. Soybeans showed little difference in time of flowering, but all other species were markedly behind in these two houses. The sunflowers of house 5 practically failed to flower, producing only a few rudimentary flowers 71 days after the first flowers appeared in house 3.

TABLE 7. *Time of First Appearance of Flowers*  
(Arranged in the Order in which the First Flowers Opened in the Different Houses)

House No. and Plant	Date of Flowering	Days from Planting	Days in House	Days behind First	House No. and Plant	Date of Flowering	Days from Planting	Days in House	Days behind First
3 Tomato....	Sept. 16	52	21		1 Soyb....	Oct. 1	36	36	
2 Tomato....	Sept. 24	60	29	8	2 Soyb....	Oct. 1	36	36	0
1 Tomato....	Sept. 24	60	29	8	3 Soyb....	Oct. 1	36	36	0
5 Tomato....	Sept. 30	66	35	14	4 Soyb....	Oct. 2	37	37	1
4 Tomato....	Oct. 3	69	38	17	5 Soyb....	Oct. 2	37	37	1
3 Buckwh....	Sept. 20	25	25		2 Tobacco .	Oct. 20	80	55	
1 Buckwh....	Sept. 20	25	25	0	1 Tobacco .	Oct. 21	81	56	1
2 Buckwh....	Sept. 20	25	25	0	3 Tobacco .	Oct. 25	85	60	5
4 Buckwh....	Sept. 23	28	28	3	4 Tobacco .	Oct. 27	86	61	6
5 Buckwh....	Sept. 29	34	34	9	5 Tobacco .	Nov. 3	94	69	14
3 Petunia....	Sept. 15	56	20		1 4 o'clock....	Oct. 23	58	58	
1 Petunia....	Oct. 6	77	41	21	2 4 o'clock....	Oct. 24	59	59	1
2 Petunia....	Oct. 7	78	42	22	3 4 o'clock....	Oct. 28	63	63	5
5* Petunia....	Oct. 29	100	64	44	4 4 o'clock....	Oct. 29	64	64	6
4 Petunia....	Oct. 31	102	66	46	5 4 o'clock....	Nov. 19	85	85	27
3 Sudan Gr...	Oct. 14	49	49		3 Sunflow..	Oct. 29	68	64	
2 Sudan Gr...	Oct. 21	56	56	7	2 Sunflow..	Nov. 3	73	69	5
1 Sudan Gr...	Oct. 27	62	62	13	1 Sunflow..	Nov. 4	74	70	6
4 Sudan Gr...	Nov. 4	70	70	21	4 Sunflow..	Nov. 10	80	76	12
5 Sudan Gr...	Nov. 11	77	77	28	5† Sunflow.	Jan. 8	139	135	71

\* One plant only produced 2-3 flowers which soon died and then blooming ceased.

† A few very tiny flowers were produced on one plant at this date.

Tomatoes, petunias, Sudan grass, and sunflowers flowered earliest when only ultra-violet rays were eliminated (house 3). There was little difference in time of flowering between houses 1 and 2 in spite of the difference between these two houses in light intensity.

Not only was the time of flowering delayed in houses 4 and 5, but the number of flowers produced was greatly reduced. Many of the plants produced only a few flowers. Petunias, for instance, in house 5 produced two or three flowers after being in the houses 64 days, and then ceased to bloom altogether but continued to maintain a vigorous growth. Four o'clocks also produced only three or four flowers in this house. Tomatoes, soybeans, and tobacco flowered more abundantly, but did not produce as many flowers as did the plants in the other houses.



This reduction in number of flowers resulted in a considerable reduction in fruit- and seed-production in houses 4 and 5 (table 8). Many of the flowers that did develop failed to set fruit, or if fruits were formed they were very small. The ripening of fruits in houses 4 and 5 was also greatly delayed except in the case of soybeans. These plants matured before all others, but produced only one or two seeds per pod and only a few pods. In the first three houses, on the other hand, an abundance of fruits and seeds was formed in tomatoes, buckwheat, tobacco, Sudan grass, four o'clocks, and soybeans.

Similar to the reduction in number of fruits produced in houses 4 and 5 was the development of food-storage organs. Such plants as carrots and four o'clocks, which ordinarily produce large storage roots, failed to do so in these houses (table 8 and Pl. XLVI, fig. 3). In the absence of only the ultra-violet part of daylight, however (house 3), these plants had the heaviest roots of the whole series. Microchemical tests of the stems of soybeans, tomatoes, tobacco, and sunflowers also showed much less storage of starch in these plants in houses 4 and 5 than in the other houses. The general weakness and succulence of the stems in houses 4 and 5 also indicated this. This failure in the development of food-storage organs was also shown in a preliminary series of experiments in which beets, radishes, and potatoes were grown in the different houses. In houses 4 and 5 potatoes failed to develop tubers, and the roots of beets and radishes were long and slender.

TABLE 8. *Fresh Weight of Plants*  
(Grams)

Plant	House No.	Fresh Weight per Plant			No. Plants Averaged	Days from Planting	Days in Houses	Remarks
		Tops	Roots	Fruit				
*Carrots. ....	1	77.47	131.40		4 pots	139	139	Tops drying
" ....	2	139.10	124.75		4 pots	139	139	Tops green
" ....	3	174.65	137.80		4 pots	139	139	Tops green
" ....	4	87.55	65.77		4 pots	139	139	Tops green
" ....	5	29.76	14.03		4 pots	139	139	Tops green
Four o'clocks.	1	57.00	103.75		2	137	137	1 plant only for tops; mature
" " .	2	107.50	106.00		2	137	137	Mature
" " .	3	98.00	142.50		2	137	137	1 plant only for tops; mature
" " .	4	100.00	80.00		2	137	137	Still green
" " .	5	16.75	8.25		2	137	137	Still green
Buckwheat. .	1	15.04		2.26	7	87	87	Fresh weight is for time of setting seed (42 days)
" ....	2	12.41		2.01	6	87	87	
" ....	3	10.74		2.12	7	87	87	
" ....	4	6.09		0.53	7	87	87	
" ....	5	2.42		0.09	3	87	87	
Tomato. ....	1	765.50		440.00	1	122	91	8 green fruits
" ....	2	870.50		481.70	1	122	91	10 green fruits
" ....	3	767.50		362.50	1	122	91	10 green fruits
" ....	4	618.50		179.50	1	122	91	4 green fruits
" ....	5	658.00		278.00	1	122	91	6 green fruits

\* All weights of carrots are per pot.

## Fresh and Dry Weight

In tables 8 and 9 are given the fresh and dry weights of some of the plants grown in the different houses. Almost without exception both the fresh weight and the dry weight of the plants as a whole or of any part of the plants were lowest in houses 4 and 5. With the exception of the soybeans, the percentage of moisture in the plants in these two houses was greater than that of the plants in the other houses. The lower percentage of moisture in the soybeans resulted from the fact that these plants were drying out and were more mature at the time of sampling, all samples having been taken at the same time rather than at the same state of maturity. Their dry weight was considerably lower, however, than that of the plants in the other houses.

TABLE 9. *Fresh and Dry Weights of Plants Analyzed*

Plant	House No.	Fresh Wt. Tops per Plant (Grams)	% Moisture	Dry Wt. per Plant (Grams)	No. Plants Averaged	Days from Planting	Days in Houses	Condition when Sampled
Sudan grass.	1	18.69	71.35	5.35	16	92	92	Seeds forming
" "	2	21.28	74.77	5.37	12	92	92	Seeds forming
" "	3	20.27	72.30	5.61	13	92	92	Seeds forming
" "	4	17.31	78.83	3.66	17	92	92	Flowering
" "	5	20.53	81.70	3.76	30*	92	92	Flowering
Sunflower...	1	181.20	85.34	26.56	2	96	92	Full bloom
" ...	2	174.30	88.46	20.11	2	96	92	Full bloom
" ...	3	163.50	87.93	19.73	2	96	92	Full bloom
" ...	4	64.80	90.65	6.06	4	96	92	No flowers
" ...	5	4.32	91.67	0.36	14*	96	92	No flowers
Tobacco....	1	204.00	89.90	20.60	1	67	42	Buds forming
" ....	2	249.60	89.94	25.10	1	67	42	Buds forming
" ....	3	213.70	89.89	21.60	1	67	42	Buds forming
" ....	4	176.40	91.43	15.10	1	67	42	Buds forming
" ....	5	152.00	91.45	13.00	1	67	42	Buds forming
Tomato....	1	765.50	89.94	77.01	1	122	91	Green fruits
" ....	2	870.50	90.11	86.09	1	122	91	Green fruits
" ....	3	767.50	89.13	83.43	1	122	91	Green fruits
" ....	4	618.50	89.33	66.00	1	122	91	Green fruits
" ....	5	658.00	91.02	59.09	1	122	91	Green fruits
Soybean....	1	11.23	74.90	2.82	24	91	91	Green pods
" ....	2	9.65	73.30	2.58	18	91	91	Green pods
" ....	3	10.81	72.20	3.01	18	91	91	Green pods
" ....	4	6.48	69.40	1.98	28	91	91	Ripe pods
" ....	5	2.90	67.05	0.86	34	91	91	Ripe pods

\* The greater number of plants here was necessary in order to obtain a sufficient sample for analysis.

There was little difference in the first three houses in fresh or dry weight of plants. Tobacco, four o'clocks, tomatoes, and Sudan grass had the greatest fresh weight in house 2, which transmitted the greater part of the spectrum of daylight. Carrots, petunias, sunflower, and coleus had the

greatest fresh weight in house 3, which eliminated only ultra-violet rays. In no case were the differences very significant.

There was apparently no relation between height of plants and total fresh or dry weight. This is strikingly shown in the growth of soybeans in house 5 as compared with those in houses 1 to 3. House 5 had the tallest plants, yet the dry weight per plant was less than one third that of the plants in the first three houses. On the other hand, the sunflower plants of house 5 were the shortest of the series and had a dry weight which was less than one seventieth that of the plants in house 1.

On the basis of dry weight, the amount of growth made in all plants in houses 4 and 5 was decidedly less than that in the other houses, in spite of the fact that the light intensities in these houses were little different from the intensity in house 2. It is also interesting to note that there was little difference in weight of plants in house 2 as compared with that of plants in either house 1 or house 3, both of which had light intensities considerably higher than the intensity of the light in house 2. Apparently the intensity of light in houses 1 and 3 was above the maximum required by the plants for maximum increase in weight.

### Results of Chemical Analyses

The results of the analyses of different plants from the different houses are given in tables 10 to 14. In general, the plants in houses 4 and 5 had the highest percentages of total nitrogen and the lowest percentages of total carbohydrates. In most instances the soluble forms of nitrogen were particularly high in these houses, while the starch and other acid-hydrolyzable materials were much lower. Considerable variation was shown in the different species analyzed as regards the percentage of insoluble nitrogen and sugars. In sunflowers, all forms of nitrogen were much higher and all forms of carbohydrates much lower in houses 4 and 5. In Sudan grass, the percentages of sugars as well as of all forms of nitrogen were highest in these two houses. In tomatoes, dextrose was low in houses 4 and 5 but there was little difference in percentage of any other compounds determined in all five houses. This is probably to be accounted for by the facts that the fruits were included in the analyses, and that the weight of the fruits in the first three houses was about double that in houses 4 and 5. In both the leaves and the stems of tobacco, the percentage of all forms of carbohydrates was low in houses 4 and 5. There was little difference in any of the houses in the percentage of insoluble nitrogen in tobacco, but the soluble forms were much higher in the last two houses.

The percentages of nitrogen and carbohydrates in soybeans in the different houses did not conform to the general trend of these substances in the other plants. Soluble forms of nitrogen were lower in houses 4 and 5 and insoluble forms were slightly higher. The percentage of total carbohydrates was high also in houses 4 and 5. The reason for these differences,

TABLE IO. *Analyses of Sudan Grass, Entire Tops of Plants (92 Days from Planting; All in Houses 92 Days)*

House	Total Wt. of Tops	Wt. per Plant	Mois- ture	Nitrogen				Total*		Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates †	
				Insoluble		Soluble		Total*		Green	Dry	Green	Dry	Green	Dry	Green	Dry
				Green	Dry	Green	Dry	Green	Dry								
1	299.0	18.7	71.35	.23	.79	.06	.22	.29	1.01	7.88	27.51	.92	3.18	.48	1.67	9.28	32.36
2	235.3	21.3	74.77	.22	.87	.06	.23	.28	1.10	6.99	27.68	1.17	4.62	.57	2.25	8.73	34.55
3	263.5	20.3	72.30	.17	.60	.05	.18	.22	.78	6.94	25.07	.88	3.16	.64	2.32	8.46	30.55
4	294.3	17.3	78.83	.19	.91	.07	.34	.26	1.25	3.69	17.42	.93	4.39	.65	3.07	5.27	24.88
5	616.0	20.5	81.70	.20	1.09	.09	.50	.29	1.59	4.00	21.87	.99	5.38	.54	2.97	5.53	30.22

\* This is the sum of the preceding two columns.

† This is the sum of the preceding three columns.

TABLE II. *Analyses of Tomato, Entire Tops of Plants (122 Days from Planting; in Houses 91 Days)*

House	Wt. of Fruit	Wt. of Plant	Entire Plant	Mois- ture	Nitrogen						Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates †	
					Insoluble		Soluble		Total*		Green	Dry	Green	Dry	Green	Dry	Green	Dry
					Green	Dry	Green	Dry	Green	Dry								
1	440.0	325.5	765.5	89.94	.07	.69	.05	.50	.12	1.19	1.82	18.00	.27	2.58	2.02	20.09	4.11	44.78
2	489.7	380.8	870.5	90.11	.09	.88	.06	.58	.15	1.46	1.58	15.93	.22	2.54	2.05	20.43	3.85	42.75
3	362.5	405.0	767.5	89.13	.08	.78	.07	.63	.15	1.41	1.85	17.00	.30	2.82	1.87	17.16	4.02	36.98
4	179.5	439.0	618.5	89.33	.11	1.02	.05	.46	.16	1.48	2.18	19.97	.33	3.02	1.32	12.11	3.83	35.10
5	278.0	380.0	658.0	91.02	.09	.88	.07	.82	.16	1.70	1.46	16.32	.21	2.32	1.38	15.40	3.05	34.04

\* This is the sum of the preceding two columns.

† This is the sum of the preceding three columns.

TABLE 12. *Analyses of Sunflower Plants, Entire Tops of Plants (66 Days from Planting; in Houses 92 Days)*

House	Total Wt. of Tops	Wt. per Plant	Mois- ture	Nitrogen				Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrate†	
				Insoluble		Soluble		Total*	Green	Dry	Green	Dry	Green	Dry	
				Green	Dry	Green	Dry								
1	362.4	181.2	85.34	.19	1.31	.03	.23	1.54	1.82	12.16	1.02	6.96	.87	5.91	25.03
2	348.5	174.3	88.46	.21	1.77	.06	.51	1.83	1.39	12.01	.20	1.69	.38	3.28	16.98
3	327.0	163.5	87.93	.21	1.70	.06	.54	2.24	1.49	12.35	.20	1.63	.40	3.31	17.29
4	259.0	64.8	90.65	.19	2.01	.15	1.55	3.56	.93	9.91	None	None	.95	.35	10.46
5	60.5	4.3	91.67	.15	1.78	.15	1.75	3.53	.59	7.13	None	None	None	.59	7.13

\* This is the sum of the preceding two columns.

† This is the sum of the preceding three columns.

TABLE 13. Analyses of Havana Tobacco (Plants 67 Days Old; in Houses 42 Days; Flower Buds Forming)

## Leaves

House	Total Wt. of Tops	Wt. of Leaves* per Plant	Moisture	Nitrogen				Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates	
				Insoluble		Soluble									
				Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry
1	204.0	176.6	89.74	.22	2.12	.05	.49	1.91	18.58	.17	1.67	.48	4.64	2.56	24.89
2	249.6	198.7	89.95	.23	2.34	.06	.56	2.16	21.42	.17	1.64	.36	3.70	2.69	36.76
3	213.7	180.1	89.76	.24	2.21	.07	.61	1.86	17.98	.18	1.67	.46	4.52	2.50	24.17
4	176.4	158.1	91.41	.24	2.77	.09	.99	.78	9.02	.10	1.09	.32	3.79	1.10	13.90
5	152.0	126.6	91.29	.19	2.13	.09	1.06	.42	4.85	.08	.84	.20	2.26	.70	7.95

## Stems

	Total Wt. of Stems	Wt.† per Plant													
1	63.5	31.8	90.83	.11	1.15	.09	1.03	.84	9.14	.60	6.34	1.42	15.48	2.86	30.96
2	74.5	37.3	89.58	.11	1.03	.08	.78	1.22	11.71	.46	4.42	1.77	16.96	3.45	33.09
3	63.6	31.8	90.16	.11	1.14	.08	.78	1.01	10.21	.60	5.42	1.69	17.20	3.30	33.83
4	40.5	20.3	90.96	.12	1.36	.12	1.17	.82	9.09	.59	6.14	1.19	13.50	2.60	28.73
5	47.0	23.5	91.50	.08	.97	.10	1.13	.59	7.00	.33	3.94	.68	8.03	1.60	18.97

\* Leaves from one plant analyzed.

† Stems from two plants analyzed.

as compared with other plants, is to be found in the fact that the plants of houses 4 and 5 were much more mature at the time of analysis. Their seed pods were ripe and dry and the stems and leaves also were drying out, whereas in the first three houses the majority of the fruits were still green and the plants were succulent. The pods were all included in the analyses. Had the analyses been made at the same state of maturity of the plants rather than at the same time of growth, the results would probably have conformed to those with other plants. The total dry weight of the soybean plants in houses 4 and 5 was considerably less than that of these plants in the other houses. Soybeans were the only plants that had a lower percentage of moisture in houses 4 and 5 at the time of analysis.

The differences in percentages of nitrogen compounds and carbohydrates in plants grown in the absence of only ultra-violet rays (house 3) as compared with those grown in the presence of these rays (houses 1 and 2) were so slight as to be negligible. In some cases the highest total carbohydrates were produced in house 2 (Sudan grass and tobacco leaves). In other cases the highest total carbohydrates were produced in house 1 (tomato and sunflower), while in soybeans and tobacco stems carbohydrates were higher in house 3 than in the first two houses. Total nitrogen was higher in house 2 than in house 3 in soybeans, Sudan grass, and tomatoes, but lower in sunflowers, tobacco, and buckwheat. In no case were the differences very significant.

In general, the differences in the composition of the plants grown in the different houses were not so great as might be expected from the differences in growth habits displayed by the plants, particularly by those grown in the last two houses. There seems to be a greater reduction in absolute amounts of all substances formed rather than a marked change in the relative percentages of the different compounds as determined by analysis.

#### DISCUSSION

The results of this investigation indicate that plants require the rays in the blue-violet end of the spectrum for good, vigorous growth. Absence of all wave lengths shorter than  $529 \mu\mu$  resulted in a condition of plants that is similar in many respects to that obtained when plants are grown in darkness or in light of very low intensity. Thus, there was at first a rapid elongation of the stem in practically all plants, and in many this continued to such an extent as to produce weak, spindly plants. There was little differentiation of tissues; strengthening tissues failed to develop well, and the plants were unable to remain erect unless supported. Leaves, though of normal size and containing an abundance of chlorophyll, were usually thin and curled or rolled. Their tissues were also less differentiated and loosely arranged. The removal of all wave lengths shorter than  $427 \mu\mu$  yielded similar results though to a lesser degree.

We have in this case, therefore, a type of etiolation caused by quality of

TABLE 14. *Analyses of Soybeans, Entire Tops of Plants (91 Days from Planting; in Houses 91 Days)*

House	Total Wt. of Plants	Wt. of Pods	Total Wt. per Plant	Mois- ture	Nitrogen						Acid-hydrolyzable Material		Sucrose		Dextrose		Total Carbohydrates	
					Insoluble		Soluble		Total*									
					Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry
1	205.7	31.0	11.43	74.90	.81	3.23	.37	1.47	1.18	4.70	4.51	17.95	.66	2.63	.29	.86	5.46	21.44
2	173.6	19.2	9.64	73.30	.64	2.36	.37	1.39	1.01	3.75	4.06	15.21	.50	1.86	.30	.99	4.86	18.06
3	194.5	23.1	10.81	72.20	.63	2.27	.40	1.40	1.03	3.68	4.95	17.79	.65	2.24	.65	2.34	6.25	22.37
4	181.5	31.5	6.48	69.40	.85	2.78	.25	.82	1.10	3.60	6.82	21.21	.87	2.79	.29	1.10	7.98	25.10
5	98.7	30.7	2.90	67.05	.91	2.74	.22	.66	1.13	3.40	5.58	16.92	1.02	3.08	.28	1.08	6.88	21.08

\* This is the sum of the preceding two columns.

Note: The plants in houses 4 and 5 had many ripe seeds and were more mature at time of sampling than the others. Others had only green pods.



light rather than by decreased light intensity. That it was indeed the quality of light and not its intensity that produced this etiolation is indicated by the fact that the plants grown in house 2, which transmitted practically all the rays of the spectrum of daylight, did not have any of the characteristics enumerated, in spite of the fact that the total light intensity in this house was at all times less than that in house 4 and not much greater than that in house 5. Furthermore, there was little difference in general growth and height of the plants in house 2 as compared with those in house 1 in which the intensity was almost twice as great but the quality was about the same. In fact, in some cases the plants of house 2 were even shorter than those of house 1 and had a somewhat greater dry weight. This fact would indicate that the intensity of light in house 2, and therefore also of that in houses 4 and 5, was above the maximum requirement of the plants, and hence any increase in intensity of the light in houses 4 and 5 could not be expected to be able to overcome the characteristics of growth which the plants in these houses manifested.

It has been shown rather conclusively (Timiriacheff, 11; Lubimenko, 8, and others) that photosynthesis proceeds at a more rapid rate in the red end of the spectrum than it does in the blue-violet end. In fact, the careful work of Ursprung (12) has shown this to be true even when regions of equal energy value in the red and blue-violet regions are used. Since, in the present work, the relative intensity of the red end of the spectrum did not vary greatly in the different houses, and since the total intensity was also nearly the same in houses 2, 4, and 5, we should expect to find the rate of photosynthesis not greatly different in these houses. The percentage of carbohydrates produced, however, was uniformly lower in the houses which eliminated the entire blue-violet end of the spectrum (houses 4 and 5), markedly lower in sunflowers and the leaves of tobacco, and the dry weight was greatly reduced. Hence we must conclude either that the rate of photosynthesis was reduced, or, what is more likely from the nature of the growth the plants made, that catabolic activities were increased and that much of the food made was immediately consumed. The general failure in the development of storage organs in houses 4 and 5 and the weakness in flower- and fruit-development lend weight to this viewpoint. Furthermore, Green (3) has shown that violet and ultra-violet rays are deleterious to diastase in the leaf, while red and infra-red rays are beneficial. Agulhon (1), Chauchard and Mazoue (2), and others have shown that the short wave lengths are destructive to many other enzymes *in vitro*, although they attributed this effect largely to ultra-violet rays. If the rays in the blue-violet end of the spectrum are destructive to enzymes in the plant, the elimination of these rays should result in increased enzym action and greater catabolic activity. Actual tests in the leaves must be made, however, before the matter can be definitely settled.

The most etiolated plants of all the species grown were the sunflowers in

houses 4 and 5. These plants also showed the greatest decreases in dry weight and in percentage of carbohydrates formed. The percentage of soluble forms of nitrogen was also decidedly higher in these plants.

It is clear from the results previously stated that the removal of the blue-violet end of the spectrum down to  $529\ \mu\mu$ , or even to  $427\ \mu\mu$ , is detrimental to the plant. Schanz (9), as a result of his work, concluded that the short wave lengths of sunlight, and particularly ultra-violet rays, are detrimental to plants. His conclusion, however, was based on the fact that he obtained taller plants in many cases when these rays were eliminated. In the present work, taller plants were also obtained in some species in the absence of only ultra-violet rays as well as in the absence of the entire blue-violet end of the spectrum, but the total growth and weight made in the former case was no greater and in the latter case was much less than that of plants grown in the presence of these rays. This is well shown in the case of the soybeans, in which the tallest plants of the series, namely, those grown in the absence of the entire blue-violet end of the spectrum, had less than a third the dry weight of the shortest plants, which were grown in the full spectrum. In house 3, which eliminated only ultra-violet rays, the only observable difference in the plants was a slightly increased height and a somewhat earlier flowering in some species. There was certainly not enough difference in the plants to warrant the recommendation of glass that eliminates ultra-violet rays for greenhouses, as was done by Schanz. Furthermore, in house 2, which transmitted practically all the ultra-violet rays found in daylight, though at a somewhat lower intensity, no detrimental effects on the plants were observed. Indeed, some of the most vigorous plants in general were obtained in this house. These results are in accord with the conclusions of Kluyver (7) that it is only the shorter ultra-violet rays, which are not present in sunlight as received on the surface of the earth, that are detrimental to plants. On the other hand, these results also indicate that ultra-violet rays are not indispensable for good, vigorous growth.

#### SUMMARY

An investigation was made to determine the effect on plants of removing definite regions of the spectrum in the blue-violet end. Several widely different varieties of plants were grown in five separate greenhouses so constructed that practically all conditions except the quality of light could be kept the same in all houses. House 1 transmitted all wave lengths from the red end to  $312\ \mu\mu$ ; house 2, all wave lengths to  $296\ \mu\mu$ ; house 3 eliminated only ultra-violet rays; house 4 eliminated all rays shorter than  $472\ \mu\mu$ ; and house 5 eliminated all wave lengths shorter than  $529\ \mu\mu$ .

General observations were made on vegetative vigor, flowering, and fruiting. The height of all plants was measured weekly. Chemical analyses and microchemical tests were made, and anatomical changes were followed in some cases. The following results were obtained:

1. When plants were grown in daylight from which all wave lengths shorter than  $529\ \mu\mu$  were eliminated, they developed the following characteristics as compared with plants grown in the entire spectrum of daylight:

- (a) An increased rate of elongation of the stem of all species during the first two or three weeks' growth; a greater final height in soybeans, tomatoes, four o'clocks, and coleus, but a decided decrease in height in sunflowers, petunia, buckwheat, and Sudan grass.
- (b) A considerable decrease in thickness of stems.
- (c) A reduction in the number of branches or side shoots.
- (d) A general curling or rolling of leaves.
- (e) Good development of chlorophyll, but a reduction in anthocyanin of leaves and flowers.
- (f) Less differentiation of stem and leaf tissues, less compact and thinner-walled cells, and a reduction in strengthening tissues.
- (g) Considerable delay in time of flowering and a reduction in the number of flowers produced.
- (h) Very weak development of seeds, fruits, and general storage organs.
- (i) Decrease in fresh weight and dry weight and an increase in percentage of moisture.
- (j) Considerable decrease in starch and total carbohydrates, and generally an increase in total nitrogen; often an increase in soluble nitrogen compounds.

The degree to which these different effects were produced varied with different species, but all species, aside from the abundance of chlorophyll, had an etiolated appearance.

2. When all wave lengths shorter than  $472\ \mu\mu$  were removed, the same effects were produced as listed above, but to a somewhat lesser degree.

3. When only ultra-violet rays were eliminated, none of the foregoing results were obtained with any of the plants used, although there was a small increase in length of stems in all species except buckwheat, as compared with plants receiving these rays. Tomatoes, petunias, Sudan grass, and sunflowers bloomed somewhat earlier than they did under any other conditions. In general, there was very little difference between plants that received all the rays of the spectrum of daylight and those from which only ultra-violet rays were eliminated.

The results obtained with plants from which all wave lengths shorter than  $529\ \mu\mu$  or  $427\ \mu\mu$  were eliminated are somewhat similar to those obtained when plants are grown under greatly reduced light intensity. That light intensity was not an important factor in the present experiment is proved by the fact that normal, vigorous growth was obtained when the plants received the full spectrum of daylight at an intensity that was at all times lower than that of the house in which all wave lengths shorter than  $427\ \mu\mu$  were removed, and only slightly greater than that of the house in which wave lengths shorter than  $529\ \mu\mu$  were eliminated.

The results as a whole indicate that, while ultra-violet rays are not indispensable, the blue-violet end of the spectrum is necessary for normal, vigorous growth of plants.

The writer is much indebted to Dr. William Crocker for placing the facilities of the Boyce Thompson Institute at his disposal and for valuable suggestions and criticisms during the progress of the work; to Mr. J. M. Arthur and to Dr. Sophia Eckerson for constant interest and assistance; to Mr. James E. Webster for the chemical analyses; and to Dr. H. H. Kimball of the U. S. Weather Bureau for supplying the relative intensity curves for the various wave lengths of the spectrum of daylight, and to many other members of the Boyce Thompson Institute for helpful suggestions. He is also indebted to Dr. Charles A. Shull for his criticism of the manuscript.

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## EXPLANATION OF PLATES

In all the figures in the following plates in which "house 6" plants are shown, the reference is to plants grown in the open and used merely for comparison.

## PLATE XLV

FIG. 1. Tomatoes from houses 1 to 6, 96 days from time of planting; when plants had been in houses 65 days.

FIG. 2. Four o'clocks from houses 1 to 6, 65 days from time of planting; when plants had been in houses 65 days.

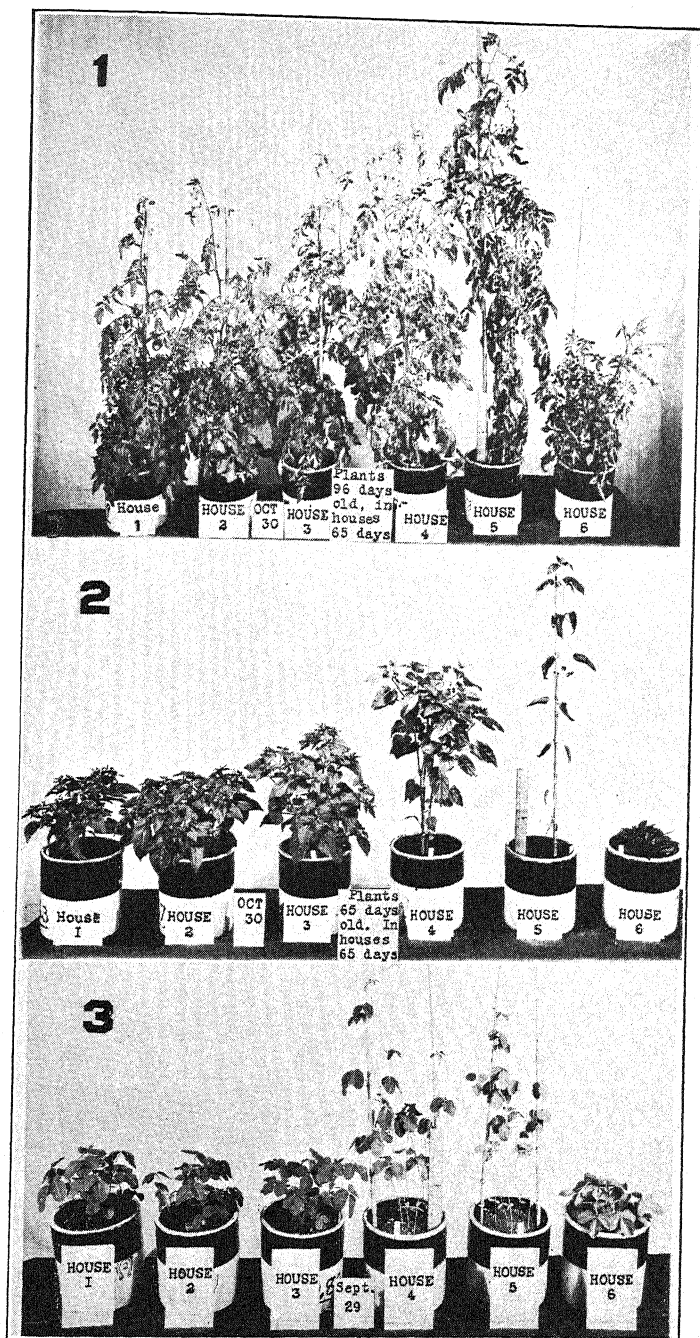
FIG. 3. Soybeans from houses 1 to 6, 34 days from time of planting; when plants had been in houses 34 days.

## PLATE XLVI

FIG. 1. Sudan grass from houses 1 to 5, 76 days from time of planting; when plants had been in houses 76 days.

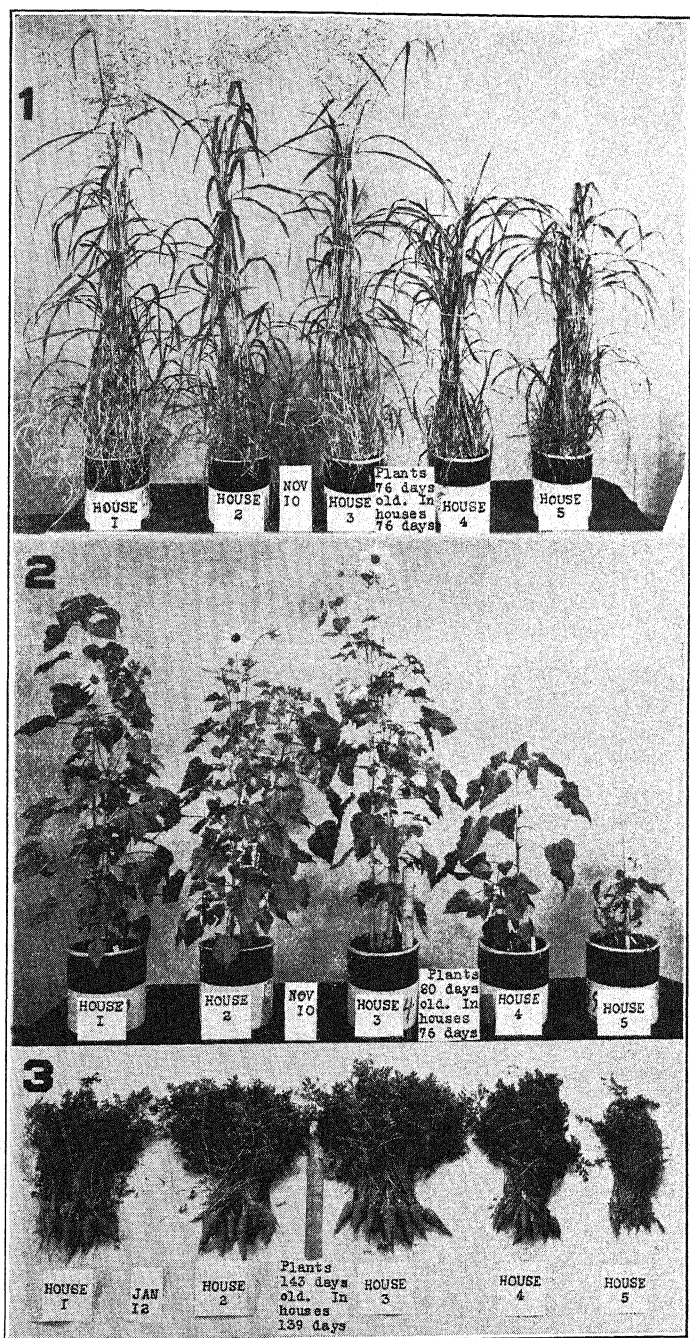
FIG. 2. Sunflowers from houses 1 to 5, 80 days from time of planting; when plants had been in houses 76 days.

FIG. 3. Carrots from houses 1 to 5, 143 days from time of planting; when plants had been in houses 139 days.



POPP: LIGHT OF VARIOUS WAVE LENGTHS





POPP: LIGHT OF VARIOUS WAVE LENGTHS





## SEED GERMINATION IN THE GRAY BIRCH, *BETULA POPULIFOLIA*<sup>1</sup>

FREEMAN WEISS

(Received for publication October 9, 1926)

The ability of *Betula populifolia* Marsh. rapidly to colonize abandoned fields and waste land is suggested in the names "old field" and "poverty" birch. In the anthracite coal fields of eastern Pennsylvania this pioneering habit is of at least some aesthetic value in initiating the forest succession on the unsightly areas that are laid waste in mining operations. Since the gray birch is regarded by foresters as a valuable nurse tree, this primary stage in the reestablishment of the forest, which here occurs without the intervention of a weed or any other successional phase, may also be of practical interest.

The areas thus denuded would appear to be extremely inhospitable even for the prevailing xeric and acid-tolerant vegetation which, since the virtual destruction of the climax forest, covers the ridge tops of the region. In strip-mining the soil is removed down to solid rock over areas from several to many acres in extent, and the subsoil is thickly deposited over the surface soil which supported the forest. Some of the rock is pyrite-bearing and gives rise to a more acid substratum than is ordinarily considered within the tolerance of green plants. In particular, the coal washings from the breakers inundate many of the swamps and small stream valleys with the result that much of the plant life is destroyed by direct sedimentation or by the toxic action of the mine water. Though this sort of denudation is strictly local, the aggregate effect is hardly negligible. Over 3 billion long tons of anthracite have been mined from this region since 1807, and in general, one half ton of rock and soil is removed for each ton of coal.<sup>2</sup> It may be seen, then, that from 1 to 2 billion cubic yards of waste material has been deposited over the land surface within the narrow limits of the anthracite fields, occupying only about 3000 square miles. If this were deposited in a layer one yard in thickness it would suffice to cover over 200,000 acres or about 300 square miles. Of course no such proportion of the land surface is covered, as most of the culm is piled in mountainous banks. However, the data give some idea of the magnitude of the surface that is to all appearances more suitable for the colonization of gray birch than for that of any other

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research. Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

<sup>2</sup> I am indebted to Mr. Daniel T. Pierce, vice-chairman of the Anthracite Operators' Conference, New York, for these data.

plant, and at least some stage of the succession bears this species predominantly.

What factors underlie the peculiar rôle played by the gray birch in this succession can only in part be inferred, *viz.*, the abundant production and thorough dissemination of its seed. Strong germination and high survival despite the intense insolation and seemingly precarious water supply of these bare mineral surfaces must be additional factors. Tolerance of high acidity is also important, since birch seedlings are found in pure stands in soil of pH 4 and lower, where huckleberries, blackberries, and sweet fern only tardily become established and probably only after the acidity is considerably reduced by drainage.

Germination records made by foresters would indicate that the characteristic abundance of birch reproduction is due to prolific seed-bearing and not to high germinative capacity. Toumey<sup>3</sup> cites Prussian records of germination only from 20 to 34 percent, and in his own tests reports 21 and 26 percent respectively for paper and cherry birch, with no sound seed remaining ungerminated after 100 days.

Helms and Jörgensen<sup>4</sup> observed that fresh seed of *Betula alba* L., or that preserved in dry conditions, will not germinate below 20° C., whereas that which has passed the winter on the forest floor will germinate at 10°.

Because of the interesting ecological problem afforded by *B. populifolia*, and because nurserymen and foresters are interested in the problem of seed germination in other birches of greater horticultural value, advantage was taken of a large collection of gray birch seed made at Freeland, Pennsylvania, in 1925, to carry out the study reported here. The seeds were stored dry at room temperature until January, 1926. The Davis catalase test then showed that they were viable, and an examination disclosed that 80-85 percent contained normal-appearing embryos.

Development of the ovules is slow following anthesis, which occurs early in May, and by midsummer only a minute embryo, which is apparently incapable of germination, is to be found. Toward the end of August the aments turn from green to brown and the bracts and samaras begin to loosen. The developed embryo then fills the carpel. Shedding of seed continues during the winter, the most persistent aments not being finally disintegrated until spring. Natural overwintering of the seed thus occurs mainly on the ground, but partly on the tree. A test limited to collections from two trees indicates that the seed is unable to germinate before it is fully ripe, that is, while the aments are partly green, as in early September; also that from collections made at full maturity, only low germination—at least no higher than that obtainable after a few months of dry storage—may be secured.

<sup>3</sup> Toumey, J. W. Seeding and planting in the practice of forestry. New York, 1916.

<sup>4</sup> Helms, Anna, and Jörgensen, C. A. Birkene paa Maglemose. Bot. Tidsskr. 39: 57-134. 1925. (From abstr. in Bot. Abstr. 15: 701. 1926.)

The behavior of seed subjected to dry storage for 4 months after its collection is shown in the following results from a test conducted at 32° C. At 25° germination was less than 10 percent, and at 20° almost nil.

TABLE 1. *Percent Germination on Successive Dates; Started Jan. 29; in Sand*

Date.....	Feb. 3	5	8	12	15	19	Mar. 1	10	Total
Percent germination.....	16	6	12	—	1	1	3	—	39
" " .....	17	3	7	4	1	3	—	1	36
" " .....	5	8	12	5	—	1	3	—	34
" " .....	12	6	12	—	—	1	—	1	32

Various treatments applied to the seeds before sowing hastened or improved germination, but the effect was not marked in any case. Low concentrations of sulfuric acid, from  $10^{-3}$  to  $10^{-5}$  normal, whether used for a prolonged immersion before sowing, or instead of water to moisten the substratum, were somewhat accelerating. Higher concentrations were injurious, as was immersion in strong acid for over 1 minute even when followed by rinsing. More favorable results followed immersion in  $\text{KNO}_3$  for 24 hours before sowing, the effect increasing up to a N/10 solution. Ethylene chlorhydrin and sodium thiocyanate were without marked effect either way. The use of mercuric chlorid (0.1 percent solution) and of chlorophenol mercury <sup>5</sup> as a disinfectant to overcome molds proved to accelerate germination, and in some concentrations to increase the percentage of germination. Treated seeds began to germinate often in 48 hours, seed soaked only in water usually a day later, and dry seed only after 1 or 2 days more. After 5 to 10 days the difference was much less evident. Tests with a mercury hydrosol showed a similar acceleration, which accordingly was attributed to the mercury content of the materials used for disinfection. Table 2 summarizes these results.

TABLE 2. *Effect of Mercuric Disinfectants on Germination; Dry Stored Seeds on Filter Paper, 32° C.*

Treatment	Period of Treatment (Minutes)	No. of Tests	Mean Percentage Germination
Dry.....		12	42
Water.....	15-30	15	48
$\text{HgCl}_2$ , 0.1%.....	1	6	52
	5	3	40
	10	3	42
	15	5	40
	20	6	41
	25	2	42
	30	2	28
Uspulun, 0.25%.....	1	6	44
	5	3	39
	10	3	46
	15	2	53
	20	6	49
	25	2	42
	30	2	36

<sup>5</sup> In this work the proprietary form of this compound known as "Uspulun" was used.

The percentage differences are not large and would seem of doubtful significance if repeated tests did not show rather consistently the same order. A compilation of all tests made on dry seeds or on those soaked only in water, as compared with those treated with bichlorid or Uspulun, when the exposure was not long enough to be injurious, is given in table 3. The treatments are rated numerically for each test, giving the highest award to the one resulting in greatest total germination; the ratings are then reduced to the basis of a uniform number of tests, and expressed as a percentage of the best rating.

TABLE 3. *Relative Value of Different Treatments;  
Dry Stored Seeds on Filter Paper, 32° C.*

	Dry	Water 15 to 30 min.	HgCl <sub>2</sub> 1 to 20 min.	Uspulun 1 to 30 min.
No. of tests.....	5	7	9	8
Total rating.....	5	14	19	19
Rating based on 9 tests.....	9	18	19	21
Percentage rating based on Uspulun = 100	43	86	90	100

Preliminary tests indicated that germination was best at slightly above 30° C. and that it declined rapidly both above this point and below 28°. Data obtained later are summarized below.

TABLE 4. *Effect of Temperature on Germination*

(a) Germination medium sand or peat, covered

Temp., ° C.....	15		20		25		32		10-32 (Alternating)	
	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat	Sand	Peat
Percent germination	0	0	0	4	2	13	26	68	26	14

(b) Germination medium filter paper, not covered

Temp., ° C.....	20-26 (Variable)						10-32 (Alternating)	
	25	28	32	36	38			
Soaked in water 15 min.....	3	5	28	40	6	0	25	
Soaked in Uspulun 0.25%, 20 min.....	12	10	44	40	4	0	43	

No consistent differences in germination obtained in granulated peat or sand or on filter paper were noted; for reasons of expediency, therefore, the procedure was generally followed of placing the seed on moist filter paper in Petri dishes. A covering of filter paper greatly reduced germination.

The high optimum temperature for germination of seeds kept in dry storage is worthy of special note in view of the behavior of seeds subjected to stratifying at low temperature now to be shown.

Samples of about 16,000 seeds were placed in after-ripening conditions on March 3, 1926, by mixing them with thoroughly moistened granulated

peat and storing them in constant-temperature chambers at 0°, 5°, and 10° C. The peat was not neutralized and was of about pH 4.6. One lot at each temperature was treated for 15 minutes in a 0.25 percent solution of Uspulun. After 1 month, and at regular intervals thereafter, samples of 50 seeds were withdrawn for germination tests. These were made usually on top of filter paper in petri dishes, but peat also was used. Later additional lots were stratified to test the effects of different concentrations and periods of exposure to Uspulun and other disinfectants. The results are summarized in tables 5-7.

TABLE 5. *Effect of Disinfection of Seeds before Stratification on Germinative Capacity (Mean Percentage Germination in 6 Tests)*

	Temperature During Germination, ° C.			
	15	20	25	30
Untreated.....	46	61	58	59
Treated with 0.25% Uspulun, 15 min.....	41	64	71	68

It is to be seen that a distinctly higher percentage of germination is secured from after-ripened seeds than from those kept in dry storage; that disinfection prior to stratification in general improves germination; and that there has been a downward shift in the temperature requirement; thus, germination of after-ripened seeds at 15° is equal to that of dry seeds at 32° C.

TABLE 6. *Effect of Temperature during After-ripening on Germination Percentage*

	Temperature During After-ripening, ° C.		
	0	5	10
Average of all tests started March 3.....	65	52	56
Average of all tests started May 3.....	46	54	54
Mean.....	55	53	55

The three storage temperatures employed seem to be equally effective in promoting after-ripening, although individual records indicate that at 0° C. the process is somewhat more rapid, at least during the first month. It appears, however, that disinfection at concentrations and exposures which do not impair germination at higher temperatures causes injury at 0°. Thus mercuric bichlorid, 0.1 percent, used on seeds subsequently stored at 0°, almost completely killed the lot, but with storage at 10° only slightly reduced germination. Uspulun at 0.5 percent caused no injury but was no more effective in improving germination than at 0.25 percent. Exposure to either concentration for 1 hour reduced germination as compared with exposure for 15 or 30 minutes.

TABLE 7. *Time Required to Complete After-ripening*  
(Percentage Germination on Successive Dates at 2 Temperatures)

Time After-ripened	Temperature During Germination, ° C.	
	20	25
30 days.....	50	51
55 days.....	62	64

An attempt to continue this observation was frustrated by germination at the storage temperature. Of the lots put in storage on March 3, those at 10° showed abundant germination *in the storage vessel* at 4 months, and those at 5° slight germination at this time. The samples stored at 0° had germinated to an amount estimated at 30 percent 6 months after placing in storage. It is of interest here to recall the practical failure of these seeds to germinate below 20–25° before after-ripening, and the observations of Helm and Jørgensen on naturally after-ripened seeds. This ability to germinate at the temperature of melting ice is doubtless a factor in the boreal distribution of birches generally, the gray birch ascending to latitude 47° in North America, and dwarf varieties of *B. pendula* penetrating the Arctic Circle.

A greenhouse planting test yielded a somewhat smaller percentage of seedlings than the germination tests promised, but showed the practicability of securing at least a 50-percent stand from fully after-ripened seed.

#### SUMMARY

The germination of *Betula populifolia* is greatly improved by storing the seeds in moist granulated peat for about 2 months at low temperature. A temperature of 10° C. is as effective for this purpose as one of 5° or 0°.

The germination percentage is increased by treating the seed with an organic mercury disinfectant before stratifying.

After-ripening results in a marked downward shift in the temperature requirement for germination.

# FACTORS INFLUENCING THE pH EQUILIBRIUM KNOWN AS THE ISOELECTRIC POINT OF PLANT TISSUE<sup>1</sup>

W. J. YODEN AND F. E. DENNY

(Received for publication September 7, 1926)

## INTRODUCTION

One of the methods used by Robbins and his co-workers (6, 7) for determining the isoelectric point of plant tissue consisted in placing pieces of the tissue in a series of buffer solutions, and in subsequently determining the change in hydrogen-ion concentration undergone by the buffer. The pH of the buffer solution that remained unchanged in  $[H^+]$  after a period of contact was designated as the *isoelectric point*. Thus, potato tissue placed in a 0.001 *M* buffer solution with pH of approximately 6.4 remained in equilibrium with the buffer, so far as  $[H^+]$  was concerned; but if the buffer was more acid than pH 6.4, its pH was changed in an alkaline direction toward 6.4, and if more alkaline than 6.4 its pH was changed in an acid direction toward the same point.

Amphoteric substances produce a similar effect upon solutions with which they come in contact; at hydrogen-ion concentrations more acid than the isoelectric point, they unite with anions, thus changing the pH in an alkaline direction; in solutions more alkaline than the isoelectric point, they unite with cations, thus changing the pH in an acid direction; at the pH of the isoelectric point they unite with neither cations nor anions and produce no change in pH (Loeb, 4).

In this respect, therefore, the tissue showed the properties of an ampholyte, and behaved in a manner analogous to that of a protein with a definite isoelectric point.

In using this method to determine the isoelectric points of a number of tissues, we obtained results which show that the equilibrium is not caused mainly by the amphoteric character of the tissue, and that the change in hydrogen-ion concentration is not due to differential absorption of ions by tissue substances that are positively or negatively charged according to the  $[H^+]$  of the solution in which the tissue is placed.

When pieces of plant tissue were placed in buffer solutions with a graded series of pH values, the equilibrium point was approximately the pH of a water extract of the tissue made under the same conditions and for the same length of time. Most of the effect upon the buffer was not due to

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research. Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.



absorption of ions from the buffer by the tissue, but was caused by substances leaching out of the tissue into the buffer. By comparing titration curves of the water extract of the tissue as originally obtained and after various treatments, it was found that the substances mainly responsible for this effect dialyzed readily through collodion, were not coagulated by heat, and were soluble in acid alcohol. This shows that proteins or other amphoteric colloids can not be the main factors in causing this change in reaction on the basis of which an isoelectric point for the tissue has been postulated.

#### THE RELATION OF THE PH OF A WATER EXTRACT OF A TISSUE TO THE SO-CALLED ISOELECTRIC POINT

In carrying out the experiments to determine the effect of a given tissue upon buffer solutions, a definite weight of tissue (usually 40 grams) was placed in a bottle, and a measured amount of a 0.002 *M* phosphate, phthalate, or borate buffer solution (usually 40 cc.) was poured on. The bottle was corked and rotated, end for end, on a turning bar. To a similar amount of tissue the same amount of water was added, and this check lot was treated in the same manner and for the same length of time. At the ends of different time intervals, a sample of liquid was removed from each bottle and the pH was determined by the quinhydrone method (1).

The potato and apple tissues used consisted of thin slices about 1 cm. in diameter by 1 mm. in thickness; the potato and barley roots were cut into pieces about 3-5 mm. long; the corn, rye, and wheat seeds were whole seeds, air-dry, except in one case in which corn-seed powder was used.

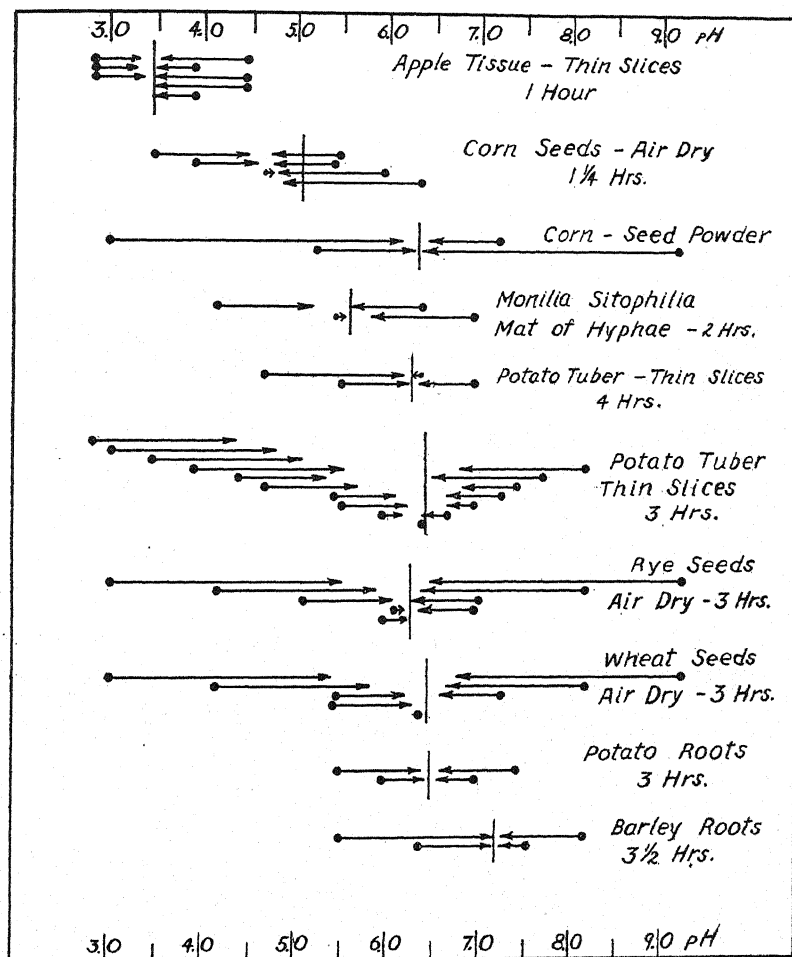
The results of these experiments are shown in text figure 1. The black dots show the pH of the buffer solution in which the sample of tissue was placed, the arrows show the direction of change undergone by the buffer, and the arrow-head shows the final pH value.

It will be noted that in all cases but one the arrows are directed toward a certain pH value, and that this value is the pH of the water extract of the tissue made under the same conditions.

Corn (*Zea mays*), air-dry seed (variety Long's Yellow Dent), as is shown in text figure 1, proved to be a possible exception among the tissues examined. The pH of the water extract of the corn seeds was not identical with the equilibrium point shown by experiments with buffer solutions. The isoelectric point found in this way after one hour's contact with buffers was always about 0.5 of a pH unit on the acid side of the pH of the water extract. When the contact was maintained for about 5.25 hours the divergence in value was reduced to about 0.25 of a pH unit. It is not known whether on prolonged contact the two values would finally become equal; bacterial action soon becomes evident if the seeds and solutions are not sterilized, and if sterilized material is used the effect of the sterilization process itself would need to be studied.

When, however, the whole corn seeds were ground to a powder, and when

samples of this powder were placed in buffer solutions and in water, the final equilibrium in the buffers was identical with the pH of the water extract of the powder, as shown in text figure 1.



TEXT FIG. 1. The relation between the pH equilibrium of tissues in a series of buffers and the pH of the water extracts of these tissues. The arrows show the pH of the buffer in which the sample of tissue was placed, the direction of change in pH, and the final pH attained. The vertical lines show the pH of the water extracts of the tissues. It is seen that the isoelectric point of the tissue as determined by this method coincides with the pH of the water extract in all tissues except in the case of corn seeds, air-dry whole seeds.

With the possible exception of whole corn seeds, therefore, the numerical value of the isoelectric point of the tissue coincided almost exactly with the pH of a water extract of the tissue. If the isoelectric point of tissue could be determined by the equilibrium point in a series of buffers, a more con-

venient method, at least for the tissues so far examined, would have been to omit entirely the use of buffers and to determine merely the hydrogen-ion concentration of the water extract of the tissue.

#### RELATION OF EQUILIBRIUM POINT TO THE HYDROGEN-ION CONCENTRATION OF PLANT JUICE

Since the so-called isoelectric point for most of the tissues examined was found to be numerically equal to the pH of a water extract of the tissue, we may inquire whether it is also identical in value with the hydrogen-ion concentration of the plant juice.

It is necessary to distinguish between the pH of the plant juice and the pH of the water extract that is obtained by bringing pieces of tissue in contact with water for certain short periods. In some cases these two values are nearly equal; thus, in the case of apple, the press-juice gave a pH of 3.39 and the water extract of the tissue after 1 hour's contact gave a pH of 3.44. In the case of potato the corresponding values were: juice = 6.10, water-extract = 6.41. With field corn, however, whole seeds rotated for 1 hour gave a water extract whose pH was 4.95, but if the seeds were ground to a powder the water extract after one hour's treatment had a pH of 6.31.

The hydrogen-ion concentration of the juice of the plant influenced the equilibrium point of the tissue in a series of buffers; but an important factor may be the rate of leaching for different substances, depending on the size of the pieces used and upon their porosity. It is likely that for powdered or porous tissues the pH of the juice of the tissue, of the water extract of the tissue, and the isoelectric point as shown by the equilibrium pH will be found to be approximately the same.

#### COMPARISON OF THE EFFECT OF THE TISSUE ITSELF AND OF A WATER EXTRACT OF THE TISSUE UPON THE REACTION OF A BUFFER SOLUTION

In order to determine the amount of change in reaction of the buffer solution produced by the tissue alone, the total change caused by tissue plus extract was compared with the change caused by extract only.

Forty grams of potato tissue were placed in each of 4 large test tubes; to 2 of these buffers were added, 1 buffer being on the alkaline side of the isoelectric point and the other on the acid side. In order to keep the volume relation comparable, 1.6 cc. of a 0.05 *M* buffer solution was added to 38.4 cc. of water; this made the solution 0.002 *M* in concentration; the pH of the diluted solution in each case is shown in column 2, table 1. To the second pair of test tubes 38.4 cc. of water only was added; the test tubes and contents were then rotated for 1 hour and the liquid was poured off; the corresponding amounts of the buffer solutions were then added to the two tubes containing the water extracts; the liquids poured off were made up to

40 cc. by rinsing with small portions of water; the final pH values were then determined.

TABLE I. *Comparison of Effectiveness of Potato Tissue and of Tissue Extracts upon the Change in pH of Buffers*

Tissue or Extract	Original pH of Buffer	Final pH of Buffer	Equivalent Amount of Acid or Alkali Required for Observed Change in pH	Extract Tissue $\times 100$
Tissue.....	5.49	6.21	2.14	
	7.64	6.66	4.30	
Water extract.....	5.49	6.17	2.08	97
	7.64	6.90	3.30	77
Water extract boiled and filtered.....	5.49	6.22	2.16	100
	7.64	7.12	1.90	44
Substances soluble in acid alcohol.....	5.49	6.08	1.92	90
	7.64	7.24	1.60	37
Substances precipitated by $\text{AgNO}_3$ .....	5.49	5.85	1.40	66
	7.64	7.17	1.68	39
Substances passing through collodion.....	5.49	6.11	1.98	92
	7.64	7.08	2.12	49

The two series differ in one important respect: in one, the buffer had been in contact with the tissue for 1 hour; in the other, the buffer had not been in contact with the tissue itself, but only with the water extract of the tissue. The results are shown in table I, from which it is seen that the tissue changed the reaction of a buffer solution from pH 5.49 to pH 6.21, while the water extract changed the reaction of the same buffer from pH 5.49 to pH 6.17. From a titration curve of the same buffer solution were read off the amounts of alkali required to change the buffer from pH 5.49 to pH 6.21 or 6.17. The amounts required are shown in column 4, table I, from which it can be seen that on the acid side about 95 percent of the acid or alkali equivalent represented by the change in reaction undergone by the buffer was furnished by the water extract. On the alkaline side the tissue was more effective, furnishing about 25 percent of the total effect.

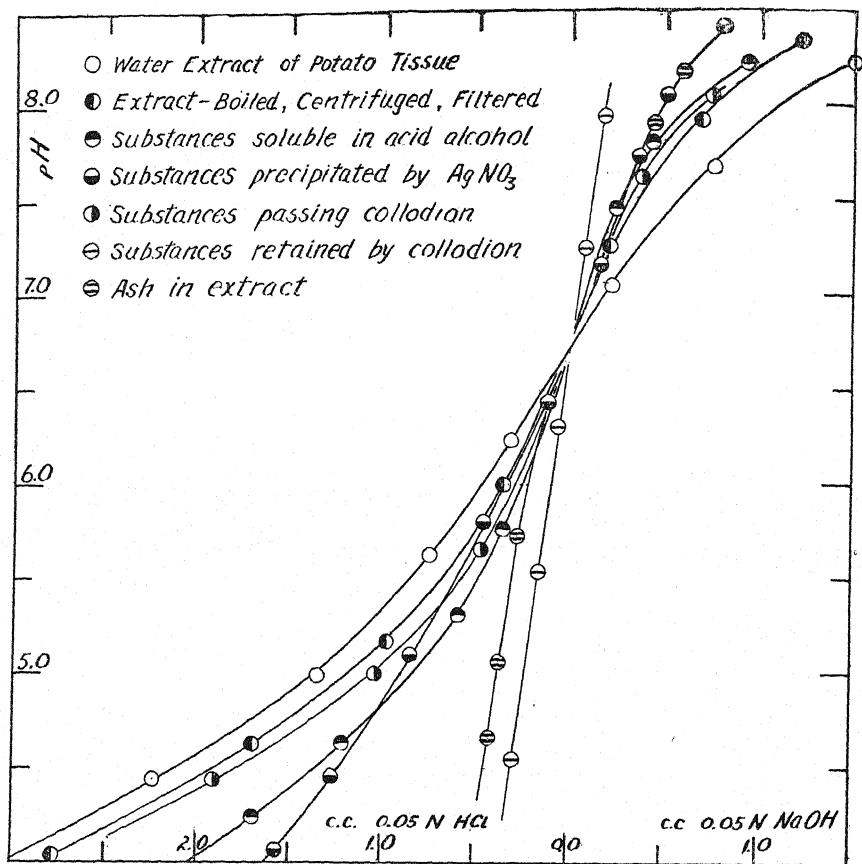
From these results we conclude that the tissue itself takes only a small part in causing the changes in the buffer solutions, that preferential absorption of ions by a tissue with a definite isoelectric point does not take place to any great extent, and that the principal cause of the change in reaction giving the appearance of an isoelectric point is related to the buffer substances that leach out from the tissue into the surrounding solution.

#### TITRATION CURVES OF THE WATER EXTRACT OF POTATO TISSUE

Since it has been shown that the substances extracted by water from the potato tissue cause most of the change in reaction of the buffer solutions coming in contact with the tissue, titration curves were made showing the

buffering capacity of the water extract (1) as obtained from the tissue and (2) after being treated in various ways. The titration curves so obtained are shown in text figure 2. In examining these curves, it should be remembered that the flatter, or more horizontal, the curve is, the greater the buffer action. A liquid having little buffer action gives almost a vertical line from pH 4 to pH 8.

From the curves shown in text figure 2, it is seen that boiling and filtering the water extract does not affect its buffer capacity on the acid side but



TEXT FIG. 2. Titration curves of the water extract of potato tissue (a) as obtained from the tissue, and (b) after the various treatments described on the graph. Note that the buffer capacity of the extract was only slightly changed by boiling and filtering, and that the principal buffer substances passed through collodion on dialysis.

causes a reduction on the alkaline side. This is also shown in table 1, column 5.

To obtain the substances soluble in acid alcohol, a measured portion of the water extract was acidified and evaporated to small volume; 95

percent alcohol was then added, and the evaporation was continued after further additions of alcohol; the mixture was then filtered and the alcohol was evaporated from the filtrate, water being added from time to time until the alcohol was completely replaced by water; the solution was then made up to the original volume. The titration curve of this extract containing the substances soluble in acid alcohol is shown in text figure 2. Much of the buffer effect was found to be present in this portion. From the data in table 1, it is seen that about 90 percent of the tissue effect on the acid side was furnished by substances soluble in alcohol; but on the alkaline side only about 37 percent of the tissue effect was attributable to these materials.

The titration curves of the materials that passed through the collodion in the process of dialysis, and of the materials that were held back by collodion, are shown in text figure 2. These are important in showing that on the acid side nearly all the buffer substances are present in the portion that passed through the collodion bag, thus showing that they are non-protein and non-colloidal.

The material in the water extract held back by the collodion (proteins and other colloids) had almost a vertical titration line from pH 4 to pH 8, showing that these materials exert almost no effect in causing the change in reaction of the buffer solutions. Other experiments showed that most of the buffer substances dialyzed through collodion within about 3 hours.

Since the principal protein of the potato, tuberin, is soluble in dilute salt solutions, and therefore may have been precipitated in the process of dialysis, a separate experiment using 5 percent NaCl for the dialysis liquid was carried out. In this case also, the material inside the collodion bag had negligible buffer capacity.

The curve marked "silver precipitate" represents the substances precipitated from the boiled water extract by acid silver nitrate. They are probably mainly organic acids. After the silver precipitate was obtained, a suspension of the precipitate in water was whirled rapidly by a stirrer, and hydrochloric acid was added drop by drop; silver chlorid was precipitated and the organic acids were set free; these were separated by filtration and the filtrate was made up to the original volume; 20-cc. portions were taken for the titration curve. This titration curve indicates that a considerable portion of the buffer action is due to organic acids present in the water extract. According to data in table 1, the substances precipitable by silver nitrate furnish about  $\frac{2}{3}$  of the buffer capacity of the tissue on the acid side, and about  $\frac{1}{3}$  of it on the alkaline side.

The graphs in text figure 2 and the data in table 1 show that, upon buffers more alkaline than the isoelectric point, the tissue itself produced an effect that was not accounted for, quantitatively, by any of the types of extracts obtained from it. Thus, in table 1, column 5, it is on the alkaline buffers that the extracts from the tissue failed to produce the amount of change in reaction caused by the tissue.

The effect of one class of amphoteric substances, the amino acids, has not been determined with exactness. The water extract of potato tissue showed an alpha-amino content of about 0.186 gram of nitrogen per liter. A solution of glycocoll containing a similar amount of nitrogen was made up, and a titration curve was prepared. This showed very little buffer action. It was considered that the rôle of amino acids in causing the change in reaction of the buffer solutions could not be studied satisfactorily until information regarding the amino acids present in the extract could be obtained.

#### BUFFER EFFECTS OF NON-AMPHOTERIC SOLUTIONS

In the preceding paragraphs the change in reaction of a buffer in contact with potato tissue is shown to be caused largely by the water-soluble buffer substances leaching out from the tissue. The capacity of a solution to cause a change in pH is described most accurately by a titration curve, and if two solutions have identical titration curves they can cause equal effects in changing the reaction of another buffer solution with which they come in contact.

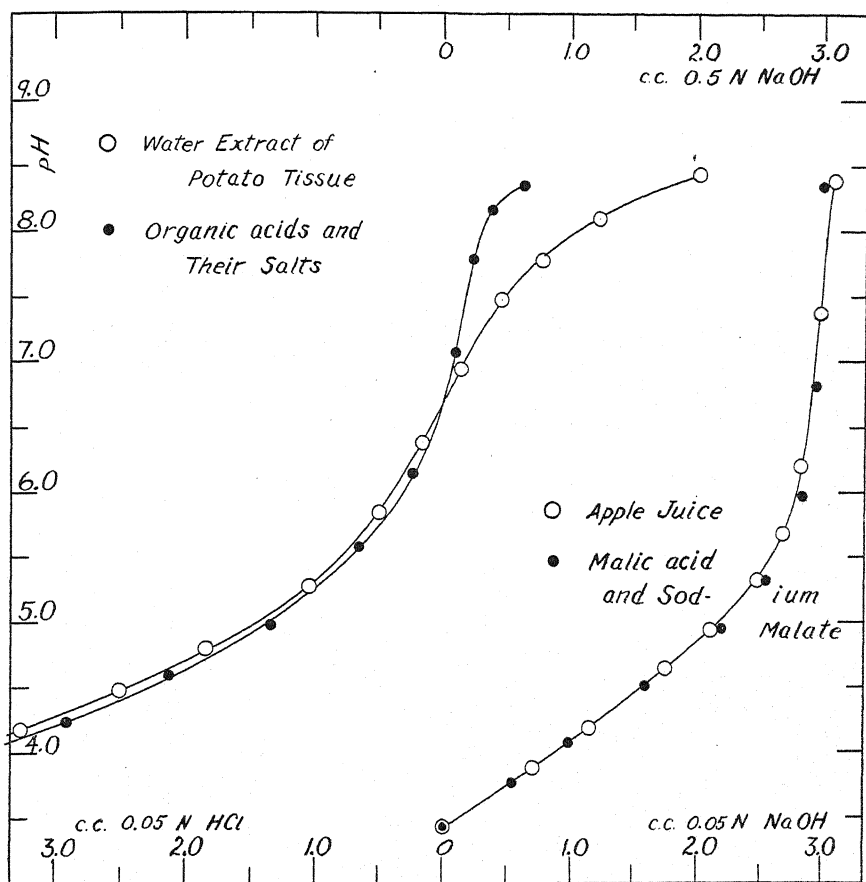
We attempted to prepare mixtures of organic acids and their salts which would give titration curves simulating the titration curves of apple juice, and of the water extract of potato tissue.

Some juice was squeezed from the pulp of Greening apples, and 20 cc. were taken for titration with  $N/2$  NaOH. The curve obtained is shown in text figure 3. To change the reaction of the juice from pH 3.43 to pH 8.39 required the addition of 3.1 cc. of  $N/2$  NaOH. An equivalent solution of malic acid was made up and found to have a pH of 2.28; sodium malate was added until the pH was 3.43, *i.e.*, equal to the pH of the apple juice. This mixture of malic acid and sodium malate was then titrated, giving the curve shown in text figure 3. The titration curves of the apple juice and of the mixture of malic acid and sodium malate were almost identical.

In another experiment, a mixture of malic, citric, oxalic, and tartaric acids having the same titration value as the water extract of potato tissue was prepared. A neutralized solution of these acids was then added to the acid solution until the mixture had the same pH as a water extract of potato tissue. This mixture was then titrated, and the titration curve is shown in text figure 3 in comparison with the titration curve of the water extract of the potato. The curves are very similar, especially on the acid side from pH 4 to pH 7. In the alkaline range the acid-salt mixture was not as well buffered as the water extract, as shown by the steepness of the curve from pH 7 to pH 8.

We do not claim that these mixtures of organic acids and salts represent the actual composition of the apple juice or potato extract. We present the data merely to show that, in order to explain the capacity of a tissue or tissue extract to cause a change in hydrogen-ion concentration in either an

acid or an alkaline direction on either side of a definite point, it is unnecessary to assume the presence of an amphoteric substance. For instance, if a portion of the artificial mixtures of organic acids and salts is added to a



TEXT FIG. 3. Similarity in the titration curves of a tissue extract or juice and of a prepared mixture of organic acids and salts. The 0.05 *N* acid and alkali refer to the potato-extract curve, and the 0.5 *N* alkali refers to the apple-juice curve.

buffer more acid than itself, the reaction will be shifted in an alkaline direction; and if added to a buffer more alkaline than itself the reaction will be shifted in an acid direction. In this respect, therefore, a mixture of organic acids and their salts behaves like a plant tissue with an isoelectric point at the pH of the acid-salt mixture.

#### DISCUSSION

It is not our purpose to claim that plant tissue does not contain substances with isoelectric points, nor that these substances are not of great



importance in the life processes of plants. Nor do we claim to have shown that the tissue itself does not have an isoelectric point. Robbins and his co-workers have brought evidence by other methods of experimentation (water absorption, toxicity of ions, staining of tissues, etc.) regarding the existence of such a point.

Our objection is mainly to the method of determining tissue isoelectric points by immersing the tissue in a series of buffers and assuming that the pH value at which no change in reaction is shown is the isoelectric point of the tissue.

Furthermore, it is unlikely that the equilibrium point of a tissue in a series of buffers represents the isoelectric point of the proteins of the tissue. Thus the equilibrium point for potato tissue is about pH 6.4; but the isoelectric point for tuberin, the principal protein of potato, is about pH 4.0 according to Cohn, Gross, and Johnson (3). Pearsall and Ewing (5) find that when the tissue is made as acid as, or more acid than, the isoelectric point of the principal protein in the tissue there is a rapid exosmosis of ions, indicating a serious injury to the tissue. The point for potato at which rapid exosmosis of chlorids took place was not at pH 6.4 but at about pH 4.4.

Chibnall (2) found that the pH of the cell contents and the isoelectric point of the cytoplasmic proteins are not identical in any tissue he studied, and points out the probability that any change in the reaction of the cell which brings the proteins to their isoelectric points will result in the death of the cell. The buffer capacity of the cell contents protects the cell against injury by tending to prevent the  $[H^+]$  from reaching the isoelectric point of the proteins of the cytoplasm.

#### SUMMARY

1. Use was made of the method of determining isoelectric points for plant tissue which consists in placing pieces of the tissue in a series of buffer solutions of varying hydrogen-ion concentrations and in noting the pH value of the buffer at which no change in reaction occurred.
2. This equilibrium pH for a number of plant tissues was found to be the pH of a water extract of the tissue in contact with water instead of buffer solution for the same time and under the same conditions.
3. Most of the effect upon the buffer solution was not due to absorption of ions from the buffer by the tissue, but was caused by substances leaching out of the tissue into the buffer. On the acid side of the isoelectric point only about 5 percent of the change in reaction undergone by the buffer was caused by the tissue itself; on the alkaline side the tissue was more effective, causing about 25 percent of the change.
4. The soluble substances which leached out of the tissue, and which exerted a dominant effect in changing the reaction of buffers in contact with the tissue, dialyzed readily through collodion, were not coagulated by heat, and were soluble in acid alcohol. This shows that proteins or other colloidal substances do not play an important rôle in causing the change in pH.

5. Since the tissue itself is not mainly involved, and since the effect produced is not due to proteins or other amphoteric colloids, it is thought that this method does not give reliable information as to the pH value of the isoelectric point of a tissue or furnish satisfactory evidence of the existence of such a point.

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# THE GERMINATION OF CENTURY-OLD AND RECENTLY HARVESTED INDIAN LOTUS FRUITS, WITH SPECIAL REFERENCE TO THE EFFECT OF OXYGEN SUPPLY<sup>1</sup>

ICHIRO OHGA

In previous papers (3, 4), I have reported the finding of very old Indian lotus (*Nelumbo nucifera*) fruits that are still alive. Thanks to their natural longevity and their impervious coats, the fruits have lain for probably more than two hundred years in moist peat in a dry, viable condition.

In the course of study of the ancient Indian lotus fruits since 1923, the writer investigated (1) the maximum and minimum amount of oxygen as related to germination and growth; (2) composition of air contained in the fruit; and (3) carbon dioxid effect on germination. The present paper is a report and discussion of these data.

## MATERIALS AND METHODS

Fruits from four sources were used in these studies: old fruits from the Pulantien Basin, South Manchuria; fruits of 1923 or 1924 from Nagoya, Japan; from Tokyo, Japan; and decorticated fruits from Shanghai, China. All showed about 100 percent germination except the decorticated fruits, which gave 44 percent germination.

Since the coats of the fruits are impervious to water, the fruits were prepared for germination by 5 hours' treatment with concentrated sulfuric acid, followed by thorough washing and later drying.

To test the relation of oxygen to the germination of the seeds, the treated fruits were placed in large-mouthed bottles filled with boiled water. The bottles were then stoppered with rubber stoppers provided with glass tubes. By proper manipulation of the tubes, the water was displaced by any desired gas. When it was desired to have the bottle practically free from oxygen, hydrogen or nitrogen gas was used for displacement, and a little vial was placed in the bottle and partly filled with potassium pyrogallate solution by an inlet tube after the boiled water had been removed from the vial by suction.

For testing the effect of known oxygen pressures on germination, treated seeds were placed in petri dishes on moist filter paper. The petri dishes were then set on tripods in large pans with a battery jar inverted over them. The pans were then filled with water, and the water was drawn to the desired height in the inverted battery jar by suction and later displaced by

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research. Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

the appropriate gas. The gas was retained in the battery jar by the water seal.

To determine the composition of the gas within the epicotyl cavity of a fruit, the coat of the fruit was partly filed away and then the fruit was immersed in a vessel of mercury in such a way that a Torricellian vacuum could be applied for the withdrawal of the gas from the fruit. The gas thus obtained was analyzed in a Bonnier and Mangin apparatus (5). Each fruit gave about 0.2 cc. of gas at atmospheric pressure and room temperature. Two to ten fruits were used for each gas sample.

In order to determine the respiratory carbon dioxide liberated from dry seeds, Pettenkoffer tubes filled with NaOH were employed (2). The tests were run at 25° C., and the carbon dioxide was determined by the double titration method of Brown and Escombe (1).

Preliminary tests at 10°, 15°, 22°, 27°, 32°, and 35° C. showed most rapid germination and growth at about 32° C. Most of the experiments were conducted near this optimum temperature.

## RESULTS

### Germination in Oxygen and Nitrogen Gas

In order to determine the limit of oxygen supply for germination, the seeds, previously treated with H<sub>2</sub>SO<sub>4</sub> to make their coats permeable to water, were placed in bottles containing the following series of gases: pure oxygen, 4 parts oxygen and 1 part nitrogen, 3 parts oxygen and 2 parts nitrogen, 2 parts oxygen and 3 parts nitrogen, 1 part oxygen and 4 parts nitrogen, and pure nitrogen. The supply of gases was renewed every day. The results of two determinations are given in table I.

TABLE I. *Lengths of Sprouts of Indian Lotus Fruits in Various Proportions of Oxygen and Nitrogen*

Proportions of O <sub>2</sub> and N <sub>2</sub>	Kind of Fruits	Series I				Series II					
		Length of Sprouts* (cm.)				Length of Sprouts* (cm.)				Average	
Oxygen .....	New	3.2	2.5	1.5	2.4	4.0	3.2	2.7	1.5	1.0	2.5
	Old	12.9	11.7	10.9	11.8	12.8	12.2	11.8	11.2	10.5	11.7
Oxygen 4 parts, nitrogen 1 part .....	New	6.0	5.5	5.0	5.5	6.5	6.2	6.0	5.7	4.8	5.8
	Old	16.2	15.9	15.5	15.9	16.7	15.8	15.3	15.0	11.5	14.9
Oxygen 3 parts, nitrogen 2 parts .....	New	5.3	5.2	2.5	4.3	5.8	5.2	4.7	4.2	2.7	4.5
	Old	14.7	11.5	5.4	10.5	15.5	15.2	12.8	11.6	8.4	12.7
Oxygen 2 parts, nitrogen 3 parts .....	New	4.5	4.0	2.2	3.8	4.8	4.6	4.0	3.6	2.0	3.8
	Old	12.6	11.5	11.2	11.8	13.5	12.2	11.6	10.5	10.2	11.6
Oxygen 1 part, nitrogen 4 parts .....	New	5.2	4.8	3.3	4.4	5.8	5.2	4.7	4.0	3.5	4.6
	Old	13.2	10.5	6.5	10.1	13.6	13.2	12.7	10.2	7.8	11.7
Nitrogen .....	New	11.8	5.5	4.9	7.3	6.0	5.5	5.2	4.8	3.5	5.0
	Old	18.2	10.9	9.6	12.9	12.0	11.2	10.8	9.7	8.6	10.5

\* After 3 days at 30° C.

The results in table 1 show that: (1) the external air had little effect on the germination; (2) the sprouts made somewhat greater growth in 4 parts of oxygen and 1 part of nitrogen; and (3) sprouts from the old fruits were more vigorous than those from new ones.

### Germination at Reduced Oxygen Pressure

The germination at reduced oxygen pressure, after a week, is shown in table 2.

TABLE 2. *Lengths of Sprouts of Indian Lotus Fruits in Reduced Oxygen Concentrations*

Proportion of Gases	Kind of Fruits	Length of Sprouts after 1 Week at Room Temperature (cm.)					Average (cm.)
Air.....	New	3.5	3.2	3.0	2.7	2.5	3.2
	Old	7.2	6.9	6.8	6.6	4.6	6.4
Air 6 parts, nitrogen 4 parts .....	New	4.5	4.3	3.7	3.5	3.1	3.8
	Old	8.7	8.2	7.2	6.5	6.2	7.4
Air 4 parts, nitrogen 6 parts .....	New	4.8	4.2	3.8	2.8	2.5	3.6
	Old	8.7	7.0	6.3	5.5	5.1	6.5
Air 1 part, nitrogen 9 parts .....	New	4.9	4.5	3.2	3.0	2.0	3.5
	Old	9.0	8.3	6.2	4.8	4.7	6.6
Water.....	New	8.5	8.2	7.8	6.0	5.0	7.1
	Old	12.2	11.5	10.2	8.5	7.4	10.0

The results in table 2 show that: (1) there were no significant differences in the germination at different oxygen concentrations; (2) the fruits germinated in moist air although fruits of most water plants will not do so; and (3) the old fruits gave more vigorous sprouts than the new ones.

### Germination in Oxygen-free Condition

Six fruits were used in each test in this series. The fruit coats were removed from one end of each of three fruits in each lot, in order to facilitate the removal of the internal air in the fruits when they were later subjected to evacuation. The bottles containing the six fruits were connected to a water-suction pump giving about 400 mm. reduced pressure for 5-10 minutes, in order to eliminate as much of the gas contained in the fruits as could be removed by this amount of suction. These bottles were then filled half with water and half with nitrogen, hydrogen, or carbon dioxide gas. The fruits were immersed about 5 cm. deep in water. One bottle was filled with tap water, and another with boiled water. After a week, the results were as shown in table 3.

The results in table 3 show that when the gas that is normally present in the fruit is removed the fruits are unable to germinate, but that no other supply of oxygen is necessary for germination except that contained inside the fruit itself.

TABLE 3. *Effect on Germination Caused by the Removal of the Gas Contained Inside the Fruit*

Treatment	Air above the Water Displaced by	Kind of Fruits	Growth of Sprouts after 1 Week (cm.)			Average (cm.)
Internal air removed * . . . . .	Nitrogen . . . . .	New	Trace	0	0	17.8
Internal air not removed . . . . .		Old	Trace	0	0	
		New	16.2	17.5	19.7	
		Old	26.5	30.7	31.2	
Internal air removed * . . . . .	Hydrogen . . . . .	New	Trace	0	0	13.6
Internal air not removed . . . . .		Old	Trace	0	0	
		New	19.2	20.5	1.2	
		Old	30.5	32.9	34.2	
Internal air removed * . . . . .	Carbon dioxide . . . . .	New	0	0	0	20.1
Internal air not removed . . . . .		Old	Trace			
		New	20.2	22.5	17.6	
		Old	35.7	30.8	32.6	
Internal air removed * . . . . .	Boiled water . . . . .	New	Trace	Trace	0	19.7
Internal air not removed . . . . .		Old	Trace	Trace	0	
		New	18.2	20.5	20.6	
		Old	30.7	32.7	31.2	
Internal air removed * . . . . .	Tap water . . . . .	New	Trace	Trace	0	19.8
Internal air not removed . . . . .		Old	Trace	0	0	
		New	17.2	20.5	21.7	
		Old	30.6	34.2	36.8	

\* About 1/5 of the fruit coat at the end from which the plumule emerges in sprouting was removed for the purpose of facilitating the removal of the internal air.

TABLE 4. *Analysis of Gas Contained in the Fruit of Nelumbo nucifera*

Kind of Fruits	Number of Determinations	Percentage of Carbon Dioxide	Percentage of Oxygen	Percentage of CO <sub>2</sub> + O <sub>2</sub>
Old . . . . .	6			19.41
	4			19.54
	4			19.41
	4			18.98
	4			19.31
	4	0.78	17.52	18.30
	4	0.76	18.52	19.28
	4	0.76	18.22	18.98
	4	0.73	18.22	18.95
	4	0.69	19.16	19.78
	42 Total	Ave. 0.74	Ave. 18.33	Ave. 19.11
New . . . . .	6			19.98
	5			19.48
	6			19.75
	3			19.71
	4	0.80	18.56	19.36
	5	0.85	18.55	19.40
	4	0.82	19.13	19.95
	4	0.76	19.40	20.16
	37 Total	Ave. 0.81	Ave. 18.88	Ave. 19.72

### Analysis of Gas Contained within the Fruit

The results of the analyses of the gases removed from the fruits are shown in table 4.

The results in table 4 show that there is little difference in the composition of gas obtained from the ancient and from the new fruits.

### Water Content of the Dry Fruits and the Rate of Respiration

The water content of the fruits was found to be as follows: old fruits 12.38 percent, new fruits (average of two determinations) 12.5 percent. A current of air was passed over dry fruits continuously for a period of 5 days. There was no measurable amount of carbon dioxid absorbed by the solution of sodium hydroxid.

### SUMMARY

1. Indian lotus fruits which had lain buried in a bed of peat probably for more than 200 years showed about 100 percent germination when the fruit coats were made permeable to water by a treatment with sulfuric acid.
2. These lotus fruits germinated well in 100 percent oxygen, 100 percent nitrogen, 100 percent hydrogen, and 100 percent carbon dioxid.
3. The length of sprouts from old fruits is always greater than that of sprouts from new ones.
4. Although they germinated thus in an external atmosphere containing no oxygen, an internal supply of oxygen was available to the embryo through the air contained in a cavity inside each fruit and in the intercellular spaces of the plant tissues. This gas contained all the oxygen necessary for the germination of the fruit.
5. The internal gas was pumped out of the fruit, collected, and analyzed. Each fruit contained about 0.2 cc. of gas. Seventy-nine analyses, when averaged, showed: (1) in the old fruits 18.33 percent oxygen, 0.74 percent carbon dioxid, and 80.93 percent nitrogen; (2) in fruits from the harvest of 1924, 18.88 percent oxygen, 0.81 percent carbon dioxid, and 80.31 percent nitrogen.
6. The respiration rate of air-dry fruits containing about 12 percent of water was so slow that a measurable amount of carbon dioxid was not obtained after three days' continuous absorption in sodium hydroxid.

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# A COMPARISON OF THE LIFE ACTIVITY OF CENTURY-OLD AND RECENTLY HARVESTED INDIAN LOTUS FRUITS <sup>1</sup>

ICHIRO OHGA

## INTRODUCTION

It was shown in a previous paper (5) that century-old and recently harvested fruits of Indian lotus differ in germination behavior, the old fruits giving more vigorous seedlings. The work here reported was undertaken to determine the relation of this fact to the rate of respiration, catalase activity, and hydrogen-ion concentration of the fruits.

## RESULTS

### Comparison of Catalase Activity of Dry Fruits

For the determination of catalase activity, a modified Appleman's (1) apparatus was used, and the amount of oxygen liberated from 10 cc. of hydrogen peroxid was used as a measure of the activity. This method was similar to that used by Crocker and Harrington (4). In preliminary tests, catalase determinations were made on fruit powder sifted through a 100-mesh screen, the amount used varying from 0.035 to 0.3 gram. A ten-minute reading was taken, and from this and from the weight of material used the catalase activity per gram was calculated. In all the experiments here reported, the activity was calculated in the same way. Table 1 shows the relation between the quantity of fruit powder used and the volume of oxygen liberated.

TABLE 1. *Catalase Activity of Lotus Fruits; Relation of Oxygen Liberated to Quantity of Fruit Tissue Used*

Weight in Grams	Cubic Centimeters of Oxygen Liberated							
	After 1 Minute		After 2 Minutes		After 5 Minutes		After 10 Minutes	
	Volume Observed (cc.)	Volume per Gram of Sample (cc.)	Volume Observed (cc.)	Volume per Gram of Sample (cc.)	Volume Observed (cc.)	Volume per Gram of Sample (cc.)	Volume Observed (cc.)	Volume per Gram of Sample (cc.)
0.30	21.0	70.0	35.7	119.0	51.2	172.0	54.5	181.0
0.15	8.5	56.5	14.5	96.5	27.6	184.0	37.3	249.0
0.07	4.5	64.5	9.0	129.0	17.5	250.0	24.1	345.0
0.035	2.2	63.0	4.3	123.0	8.1	231.0	11.7	335.0

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research. Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

As shown in table 1, the maximum liberation of oxygen took place when 0.07 gram of fruit powder was used. Although the weight of half of a cotyledon (0.3 to 0.4 gram) exceeds this, the writer in practice used half of a cotyledon for each measurement. One plumule weighing approximately 0.025 gram was used in each test. Table 2 shows the results of the measurements of catalase activity of air-dry old and new fruits.

Table 2 shows that: (1) the catalase activity of the old fruits is much higher than that of the new fruits; and (2) catalase activity of the plumule is greater than that of the cotyledon.

TABLE 2. *Catalase Activity of Air-dry Old and New Lotus Fruits; Cubic Centimeters of Oxygen per Gram of Fruit Tissue*

Old Fruits		New Fruits	
Cotyledon	Plumule	Cotyledon	Plumule
208	357	172	344
224	280	152	200
221	332	167	304
306	363	159	410
256	362	181	300
243	339	166	311

### Comparison of Catalase Activity of Soaked Fruits

The fruits were soaked in tap water at 30° C., one lot of fruits being soaked for 12 hours and another lot for 5 days. Tables 3 and 4 show the catalase activity of old and new fruits after soaking in water.

TABLE 3. *Catalase Activity of Old and New Lotus Fruits Determined after 12 Hours' Soaking in Tap Water at 30° C.; Cubic Centimeters per Gram of Fruit Tissue*

Old Fruits		New Fruits	
Cotyledon	Plumule	Cotyledon	Plumule
123	105	109	94
118	119	108	106
120	123	114	110
120	116	110	107

TABLE 4. *Catalase Activity of Old and New Lotus Fruits Determined after 5 Days' Soaking in Tap Water at 30° C.; Cubic Centimeters per Gram of Fruit Tissue*

Old Fruits		New Fruits	
Cotyledon	Plumule	Cotyledon	Plumule
147	123	117	91
165	124	119	68
170	126	146	84
161	124	127	81

The results in tables 3 and 4 show that: (1) the catalase activity of old fruits is higher than that of new ones; (2) in soaked fruits the catalase activity of the cotyledon is greater than that of the plumule. This is the reverse of the condition found for air-dry fruits.

### Comparison of Catalase Activity in Anaerobically Treated Fruits

Series I: In order to determine the catalase activity after culture in oxygen-free condition, one lot was kept in cooled boiled water, and the other, after evacuation for ten minutes with a 400-mm. water-suction pump, was kept in cooled boiled water. In the latter case, sprouts did not develop. After four days the determinations of catalase activity were made. The results are shown in table 5.

TABLE 5. *Catalase Activity of Lotus Fruits after Being Left in Boiled Distilled Water for 4 Days; Cubic Centimeters of Oxygen per Gram of Fruit Tissue*

Treatment	Old Fruits		New Fruits	
	Cotyledon	Plumule	Cotyledon	Plumule
Coat on.....	172.0	60.2	91.3	47.2
	186.5	64.9	121.0	59.0
	182.4	66.2	112.4	52.7
	180.3	63.8	108.2	53.0
Coat stripped and evacuated with water pump..	88.0	33.0	43.6	17.0
	82.0	30.5	41.0	19.5
	92.2	29.5	50.7	16.7
	87.4	31.0	45.1	17.7

Series II: In order to determine the catalase activity in oxygen-free condition, the fruits, after the treatment above described, were kept in nitrogen gas. After four days the determinations were made. The results are shown in table 6.

TABLE 6. *Catalase Activity of Lotus Fruits Left in Nitrogen Gas for 4 Days; Cubic Centimeters of Oxygen per Gram of Fruit Tissue*

Treatment	Old Fruits		New Fruits	
	Cotyledon	Plumule	Cotyledon	Plumule
Coat on.....	142.2	59.1	104.3	41.5
	158.3	63.5	123.4	52.3
	134.5	67.2	116.7	47.8
	145.0	63.3	114.8	47.2
Coat stripped and evacuated with water pump..	82.5	38.0	67.0	33.9
	95.7	42.8	70.2	35.7
	103.5	45.2	59.8	31.6
	93.9	42.0	65.7	33.7

The results in tables 5 and 6 show that: (1) catalase activity is greater in old fruits than in new ones; and (2) catalase activity is greater in the cotyledon than in the plumule.

### Comparison of Respiration Rate in Dry and Wet State of New and Old Fruits

To determine the respiration rate during germination, a current of carbon dioxid-free air was drawn through a vessel containing the fruits and then through Pettenkofer tubes containing sodium hydroxid. The carbon dioxid absorbed was estimated by the double titration method of Brown and Escombe (3). Twenty fruits were used in each test. With fruits having intact coats a 3-day test did not show a measurable amount of carbon dioxid. But when 5 fruits with sprouts about 3 inches long that had been kept 3 days in a 30° C. incubator were transferred to a respiration chamber, much larger quantities of carbon dioxid were given off. The results are given in table 7.

TABLE 7. *Respiration of New and Old Fruits of Indian Lotus*

	Milligrams CO <sub>2</sub> Liberated	
	Old Fruits	New Fruits
Twenty air-dry fruits in 3 days at 25° C.....	0	0
Five soaked 1 day at 25° C.....	.012	.009
	.015	.005
	.015	.008
	.012	.007
	.015	.006
		.007
Average.....	.014	.007

The results in table 7 show that: (1) dry fruits after 3 days at 25° C. did not yield a detectable amount of carbon dioxid; (2) soaked fruits from which the plumule had just begun to emerge liberated measurable quantities of carbon dioxid; and (3) the respiration of old fruits was more intense than that of new ones.

### Comparison of Acidity in Dry and Wet State of Old and New Fruits

In order to determine the acidity of fruits, pH value was estimated by the quinhydrone-electrode method (2). Cotyledons and plumules were tested separately, using either a half of a cotyledon weighing approximately 0.35 gram in the dry and 0.75 gram in the wet condition, or an entire plumule weighing 0.025 gram in the dry and 0.10 gram in the wet condition. The plant tissue was ground in a mortar, 10 cc. of water were added, and the supernatant liquid was used for the determination. The results obtained are shown in tables 8 and 9.

TABLE 8. *Hydrogen-ion Concentration of Old and New Fruits of Indian Lotus; Determinations Made on Dry Fruits*

Old Fruits		New Fruits			
		Series I		Series II	
Cotyledon	Plumule	Cotyledon	Plumule	Cotyledon	Plumule
6.68	6.50	6.71	6.58	6.83	6.53
6.71	6.52	6.68	6.64	6.68	6.52
6.53	6.51	6.68	6.64	6.50	6.52
6.53		6.68	6.64	6.70	
6.56		6.68	6.64	6.70	
6.60	6.51	6.69	6.63	6.68	6.52

TABLE 9. *Hydrogen-ion Concentration of Old and New Fruits of Indian Lotus; Determinations Made on Soaked Fruits*

Old Fruits		New Fruits			
		Series I		Series II	
Cotyledon	Plumule	Cotyledon	Plumule	Cotyledon	Plumule
6.48	6.54	6.74	6.75	6.53	6.59
6.54	6.58	6.59	6.75	6.53	6.59
6.54	6.63	6.59	6.75	6.59	6.75
6.41	6.63	6.70	6.70	6.59	6.81
6.41	6.66				
6.46	6.64	6.65	6.74	6.56	6.69

Tables 8 and 9 show only small differences in the pH of tissues from old and new fruits. The error of a single determination by the method used was about 0.03 pH. The differences found were about two to four times this error, and in almost every comparison showed a slightly higher acidity in the old fruits than in new ones. It is not known whether this small difference, if it exists, is an important factor in contributing to the superior vigor of the seedlings of old fruits.

#### SUMMARY

1. The growth rate of old Indian lotus fruits was greater than that of new fruits.
2. The catalase activity of dry and soaked old fruits was greater than that of new ones.
3. The plumule showed greater catalase activity than the cotyledon in dry fruits, but in soaked fruits the reverse was true. The reason for this needs further investigation.
4. The respiration rate of dry fruits could not be measured because of

the slowness of respiration. However, when sprouted fruits were tested, it was found that the respiration rate of old fruits was greater than that of new ones.

5. Only small differences in the hydrogen-ion concentration were found, nearly all measurements, however, showing a higher acidity in the old fruits.

Grateful acknowledgment is made to Dr. William Crocker for the suggestions made during the progress of this work.

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# A DOUBLE MAXIMUM IN THE RATE OF ABSORPTION OF WATER BY INDIAN LOTUS SEEDS<sup>1</sup>

ICHIRO OHGA

## INTRODUCTION

In the course of a study of Indian lotus fruits (2), I had occasion to investigate the rate of water-absorption by the seeds. When the seeds were placed in water at various temperatures, the initial rate of water intake was rapid, this being followed by a decrease, then by an increase and a later decrease. This double maximum in the rate of water-intake appeared in the result of each of sixteen tests. The purpose of this paper is to describe and discuss the experiments which led to these results.

## METHODS

Five fruits of nearly similar shape, with smooth surface and perfect seed coats, were selected for each experiment. Each lot was placed in a small beaker filled with distilled water. The beakers were placed in incubators equipped with automatic temperature regulation. The temperatures selected were 5°, 20°, and 35° C. After having soaked for certain time intervals, the seeds were taken out, the surface water was wiped off with a dry cloth, and the seeds were weighed. The whole process of removing the seeds from the water and returning them to the same water again after they were weighed was accomplished in about a minute. The time required for weighing was not deducted from the total time interval.

At the start of each experiment the seeds were weighed every 15 minutes, later every 30 minutes, and finally every hour. Although the total possible absorption percentage is about 170 percent on the basis of air-dry weight, the experiment was discontinued when the absorbed water amounted to about 100 percent of the air-dry weight.

## RESULTS

Tables 1, 2, and 3 show the percentage of moisture-intake based on the air-dry weight. The figures in columns 3, 5, 7, 9, 11, and 13 are the differences in the absorbed percentage at every half-hour or every hour interval. Text figure 1 shows the differences in percentages at every hour interval, taking the time as abscissa and percentage of absorbed water as ordinate. As will be seen both in the tables and in the figure, the rate of moisture-intake by the seeds in the first 60 minutes was high, but in every case the hourly

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research. Published, at the expense of the Institute, out of the order determined by the date of receipt of the manuscript.

TABLE I. *Water Intake by Indian Lotus Seeds*  
(Temperature, 35° C.)

Time (Min- utes)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V		Experiment VI		Ave. Percent Absorption	Ave. Rate per Half Hour
	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour	Percent Absorption	Rate per Half Hour		
15	9.78*												9.28	
30	17.12	17.12	9.89	17.31	9.04	15.76	9.42	16.18	8.74	14.81	8.82	14.97	16.03	16.03
45	23.64		17.31		15.76		16.18		14.81		14.97		21.91	
60	29.35	12.23	23.90	12.64	21.45	11.11	21.98	11.12	19.90	9.95	20.59	9.90	27.18	11.15
75	34.24		29.95		26.87		27.30		24.76		28.88		31.63	
90	38.86	9.51	35.16	10.16	30.49	7.24	32.13	8.69	32.77	8.01	32.62	7.75	35.74	8.56
105	42.93		40.11		34.11		35.99		36.41		35.83		39.80	
120	47.28	9.42	44.51	8.79	37.98	8.27	39.13	6.52	40.78	8.01	38.77	6.15	43.77	8.03
135	54.08		48.90		42.38		42.51		45.63		42.51		48.25	
150	60.87	13.59	54.12	9.89	47.29	10.85	45.89	6.52	51.70	10.92	46.79	8.02	53.40	9.63
165	67.39		58.79		53.23		49.03		57.28		50.80		58.29	
180	73.10	12.23	62.91	8.24	59.17	11.37	52.17	5.80	62.62	10.92	55.35	8.56	62.92	9.52
195	79.08		67.03		64.60		54.83		67.96		62.03		68.16	
210	84.78	11.68	71.43	8.52	70.02	11.59	58.45	7.25	74.03	11.41	67.91	12.56	73.26	10.34
225	89.67		75.55		75.19		62.08		79.61		73.26		78.10	
240	93.48	8.70	79.95	8.52	80.62	10.34	65.46	6.52	84.95	10.92	77.01	10.10	82.28	9.02
255	96.74		84.07		85.53		68.60		88.35		79.95		85.51	
270	99.46	5.98	87.36	6.32	89.41	7.49	71.26	5.07	91.02	6.07	82.62	5.61	88.36	6.08
285	102.45		90.39		93.02		73.67		93.45		84.76		90.85	
300	105.16	5.70	95.88	5.49	95.01	5.43	76.09	4.83	95.87	4.85	86.63	4.01	92.83	4.47
			95.88		98.45		78.50							

\* Percentage of water absorbed, basis of air-dry weight.



TABLE 2. *Water Intake by Indian Lotus Seeds*  
(Temperature, 20° C.)

Time (Minutes)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V		Ave. Percent Absorption	Ave. Rate per Hour
	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour		
15	6.40*		6.67		6.61		6.58		6.93		6.64	
30	11.20		11.30		11.29		11.51		12.05		11.47	
45	16.00		15.07		15.43		15.89		15.96		15.67	
60	19.47	19.47	18.26	18.26	18.43	18.43	19.18	19.18	19.58	19.58	19.04	19.04
75	22.67		21.74		21.76		22.19		23.19		22.31	
90	25.60		24.93		24.79		25.21		26.58		25.47	
105	28.27		27.83		27.55		27.95		29.82		28.28	
120	30.93	11.46	30.43	12.17	30.03	11.60	30.41	11.23	32.53	12.95	30.87	12.83
135	33.33		32.76		32.23		32.60		34.94		33.17	
150	35.20		34.78		34.43		34.79		37.05		35.25	
165	37.07		36.52		36.36		36.99		39.16		37.22	
180	39.20	8.27	38.26	7.83	38.29	8.26	39.18	8.77	41.57	9.04	39.30	8.53
195	41.87		40.58		40.22		42.47		44.28		42.00	
210	45.07		43.48		42.42		46.58		47.89		45.09	
225	48.27		46.09		44.63		51.50		51.81		48.46	
240	50.93	11.73	48.41	10.15	46.84	8.55	54.79	15.61	55.12	13.55	51.22	11.92
255	53.87		50.72		48.76		57.53		58.43		53.86	
270	56.53		53.04		50.96		60.00		61.45		56.40	
285	59.20		55.36		53.17		62.19		64.16		58.82	
300	61.87	10.94	57.39	8.98	55.10	10.26	64.38	9.59	66.87	11.75	61.12	9.90
315	64.53		59.72		57.30		66.58		69.88		63.60	
330	67.47		62.32		58.95		68.22		72.89		65.97	
345	70.13		64.64		60.88		70.14		75.60		68.28	
360	72.80	10.93	66.66	9.27	62.53	7.43	71.78	7.40	78.31	11.44	70.42	9.30
390	77.87		71.59		66.94		75.62		83.43		75.09	
420	82.13	9.33	75.94	9.28	71.07	8.54	79.18	7.40	88.25	9.94	79.31	8.89
450	86.56		79.71		75.76		82.19		92.47		83.15	
480	88.80	6.67	83.19	7.25	80.44	9.37	85.21	6.03	96.39	8.14	86.81	7.50
510	92.00		86.67		84.04		87.95		99.70		90.07	
540	95.20	6.40	89.86	6.67	87.33	6.89	90.68	5.47	102.71	6.32	93.14	6.33
570	97.60		92.75		90.36		92.88		105.42		95.80	
600	99.47	4.27	95.07	5.21	93.11	5.78	95.07	4.39	107.83	5.12	98.11	4.97
630	101.34		97.89		95.32		97.27		110.24		100.31	
660	103.20	3.73	99.42	4.35	97.52	4.41	99.18	4.11	112.35	4.52	102.31	4.20
690	105.07		101.74		99.72		101.37		114.16		104.41	
720	106.40	3.20	103.77	4.35	101.65	4.13	103.29	4.11	115.66	3.31	106.15	3.84

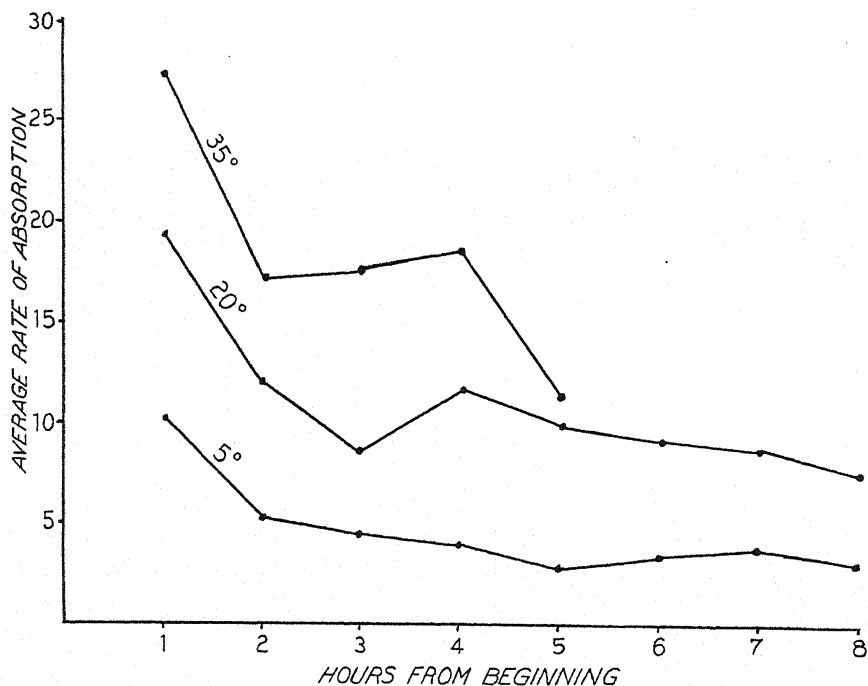
\* Percentage of water absorbed, basis of air-dry weight.

TABLE 3. *Water Intake by Indian Lotus Seeds*  
(Temperature, 5° C.)

Time (Minutes)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V		Ave. Percent Absorption	Ave. Rate per Hour
	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour	Percent Absorption	Rate per Hour		
15	3.11*		3.48		3.21		3.25		3.38		3.23	
30	5.37		6.22		5.61		6.00		6.23		5.83	
45	7.62		8.95		7.75		8.50		8.83		8.28	
60	9.32	9.32	10.94	10.94	9.89	9.89	10.75	10.75	11.16	11.16	10.41	10.41
75	11.01		12.43		11.23		12.25		12.47		11.82	
90	12.43		13.68		12.56		13.50		13.77		13.13	
105	13.84		14.92		13.63		14.75		15.06		14.39	
120	14.97	5.65	16.17	5.23	14.43	4.54	16.00	5.25	16.36	5.20	15.58	
135	16.10		17.41		15.50		17.25		17.40		16.73	5.17
150	17.23		18.65		16.57		18.25		18.44		17.82	
165	18.36		19.65		17.64		19.25		19.48		18.87	
180	19.20	4.23	20.64	4.47	18.71	4.28	20.00	4.00	20.52	4.16	19.81	4.23
210	21.46		22.38		20.58		22.50		22.60		21.91	
240	23.44	4.24	23.87	3.23	22.45	3.74	25.00	5.00	24.42	3.90	23.84	4.03
270	25.13		25.37		23.52		26.50		25.97		25.30	
300	26.55	3.11	26.86	2.99	24.32	1.87	28.00	3.00	27.28	2.86	26.60	2.76
330	28.24		28.10		25.39		29.75		28.57		28.01	
360	29.93	3.38	29.10	2.24	26.20	1.88	31.25	3.25	29.87	2.59	29.27	2.67
390	31.63		30.84		27.80		33.25		31.43		30.99	
420	33.04	3.11	32.58	3.48	29.40	3.20	35.00	3.75	32.99	3.12	32.60	3.33
450	34.74		34.97		30.47		36.25		34.55		34.07	
480	36.15	3.11	35.56	2.98	31.54	2.14	38.00	3.00	36.10	3.21	35.17	2.87
510	37.56		37.06		33.41		40.00		37.92		37.19	
540	38.70	2.55	38.55	2.99	35.01	3.47	41.75	3.75	39.48	3.38	38.70	3.23
570	40.10		39.86		36.09		43.25		40.78		40.00	
600	41.23	2.53	41.03	2.48	36.89	1.88	44.50	2.75	42.08	2.60	41.14	2.44
630	42.93		43.02		38.22		46.00		43.90		42.81	
660	44.62	3.39	45.01	3.98	39.56	2.67	47.50	3.00	45.45	3.37	44.43	3.29
690	46.03		46.51		40.36		48.75		46.49		45.63	
720	47.17	2.55	48.00	2.99	41.16	1.60	50.00	2.50	47.53	2.08	46.77	2.34

\* Percentage of water absorbed, basis of air-dry weight.

rate decreased to a first minimum, then increased to a second maximum, and then dropped to a final minimum. At  $35^{\circ}\text{C}$ ., the half-hourly rate of absorption decreased from 16.03 to 8.03 and then rose to 10.34. At  $20^{\circ}\text{C}$ . the first minimum occurred after about 3 hours and the second maximum after about 4 hours. At  $5^{\circ}\text{C}$ ., the differences in rate are not so marked as in the two former cases, but in each test a small secondary rise is shown.



TEXT FIG. 1. Average rates of absorption after various time intervals from the beginning. Ordinates are percentages on the basis of air-dry weight. Data taken from tables 1, 2, and 3.

The absorption rate gradually decreased after the second maximum of water-intake at about the time the plumule began to grow. The absorption diminished more quickly at higher temperatures. Thus, at  $35^{\circ}\text{C}$ . the plumule developed in 10 to 15 hours, at  $20^{\circ}\text{C}$ . in 20 to 26 hours, and at  $5^{\circ}\text{C}$ . it did not develop even after several weeks.

From the figure and the tables it can be seen that there appears a change in the rate of moisture-intake at about 50 percent water-absorption after 2 hours at  $35^{\circ}\text{C}$ ., at about 40 percent after 3 hours at  $20^{\circ}\text{C}$ ., and at about 30 percent after 36 hours at  $5^{\circ}\text{C}$ .

#### Experiments Relating to the Cause of the Double Maximum

*Hydrogen-ion Concentration.* Since it has been found by MacDougal (1) that the swelling of colloid material is influenced by the hydrogen-ion

concentration, measurements were made of the pH value of extracts of the seeds after different periods of soaking. Only small changes in pH were found, and these did not appear to be related in any way to the change in rate of absorption.

*Air Pocket.* There is a small air pocket in the center of each seed. In order to determine whether the second maximum in water intake is related to the infiltration of water into this pocket, the rate of absorption of cotyledons only was measured. The results are shown in table 4. The double maximum is shown in the results at 35° and 20° C., the secondary rise at 5° C., however, being small. The presence of an air pocket is not necessary for a second maximum in rate of water-intake.

TABLE 4. *Water Intake by Cotyledons of Indian Lotus Seeds*

Time (Hours)	Temperature, 5° C.			Temperature, 20° C.			Temperature, 35° C.		
	Experiment I	Experiment II	Average	Experiment I	Experiment II	Average	Experiment I	Experiment II	Average
1	23.25	22.97	23.11	33.04	35.20	34.12	34.60	33.1	33.9
2	9.80	10.81	10.31	12.46	12.72	12.59	19.2	21.4	20.3
3	8.96	9.46	9.23	11.90	11.84	11.87	23.6	25.4	24.5
4	6.50	7.84	7.17	12.16	12.42	12.29	16.7	16.8	17.1
5	6.45	6.21	6.33	11.59	13.32	12.46	12.3	8.6	10.1
6	7.84	6.49	7.17	9.28	9.76	9.52	6.0	5.8	5.7
7	6.72	5.67	6.20	9.57	8.88	9.23	5.3	4.1	4.8
8	5.89	7.03	6.46	6.37	6.84	6.61			
9	5.60	5.41	5.51	5.22	3.55	4.39			

### Double Maximum in Shull's Results with Split Pea

When *Xanthium* seeds were soaked, no double maximum was found; hence it appeared possible that the change in rate with lotus seeds was

TABLE 5. *Water-absorption by Seeds of Split Pea from Data of Shull (3)*

Time (Minutes)	Variety of Split Pea		Green Canada Field Pea		Small Scotch Yellow		Tom Thumb Yellow	
	H <sub>2</sub> O Intake	Half-hour Rate	H <sub>2</sub> O Intake	Half-hour Rate	H <sub>2</sub> O Intake	Half-hour Rate	H <sub>2</sub> O Intake	Half-hour Rate
1	4.25		4.09		5.38		3.76	
15	16.20		16.68		25.09		15.50	
30	23.84	23.84	22.98	22.98	33.69	33.69	21.15	21.69
45	30.14		28.78				26.16	
60	35.32	11.48	34.96	11.98	50.89	17.20	30.11	8.96
75	40.30		42.52		58.24		33.33	
90	45.22	9.90	50.41	16.45	69.59	18.70	37.28	7.17
105	52.06		64.76		68.10		45.34	
120	57.50	12.28	73.96	23.55	70.34	.75	51.61	14.33
135	61.42		79.48		72.58		57.21	
150	65.34	7.84	82.99	9.03			62.01	11.40
165	68.60						67.16	
180	70.32	4.98					70.74	10.73
195	72.18							
210	72.97	2.65						

related to the thickness of the cotyledons. Since Shull (3) in 1920 had measured the rate of intake of water by split pea seeds, which also have thick cotyledons, a recalculation of his data was made in order to show the half-hourly amounts of absorption. When this was done, a double maximum was found in Shull's results with split pea (table 5).

Although Shull did not use the words "double maximum" in this case, he noted this change in rate and calls attention to it in the following words (3, page 380): "After a certain critical percentage has been reached, however, they show a remarkable rise above the ideal curve indicated by the first part of the absorption."

In table 5, which is taken from Shull's paper, the half-hourly rates are shown in columns 3, 5, 7, and 9. The unknown variety of split pea shows a first minimum at about 90 minutes and a second maximum at about 120 minutes. Green Canada field pea and Tom Thumb Yellow also show second maxima, but the result with Small Scotch Yellow is doubtful.

#### SUMMARY

1. When Indian lotus seeds were soaked in water at different temperatures, there appeared a double maximum in the rate of water-intake.

2. At 35° C., the first minimum in rate occurred after about 120 minutes' soaking, and the second maximum after about 210 minutes; at 20° C. these changes in rate occurred after 180 minutes and 240 minutes, respectively; at 5° C. the differences, although less marked quantitatively, appeared after about 300 minutes and 420 minutes.

3. The changes in rate of water-absorption were not found to be correlated with change in hydrogen-ion concentration.

4. The separated cotyledons also showed a second maximum in rate of water-intake, indicating that infiltration of the internal air pocket was not a factor.

5. A recalculation of the data obtained by Shull (3) in 1920 with split peas showed second maxima in rates of water-absorption. Shull also noted this change in rate and calls attention to it in the paper cited.

6. The cause of the appearance of the second maximum is not clear. It is probable, however, that it is related to the nature and structure of the seed materials, especially to their thickness.

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